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<p>(54) Title: DETERGENT COMPOSITIONS COMPRISING ANTIBODY CONTROLLED PROTEOLYTIC ACTIVITY</p> <p>(57) Abstract</p> <p>The present invention relates to laundry detergent compositions comprising a protease and a protease-directed antibody in order to provide excellent cleaning performance and fabric care performance.</p>			

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DETERGENT COMPOSITIONS COMPRISING ANTIBODY CONTROLLED PROTEOLYTIC ACTIVITY

FIELD of the INVENTION

The present invention relates to laundry detergent compositions comprising a protease and a protease-directed antibody in order to provide excellent cleaning performance and fabric care performance.

BACKGROUND of the INVENTION

An important part of the system which protects vertebrates against infections by bacteria and viruses is the humoral immune system. Specialised cells present in bone marrow, lymphoid tissues and blood, produce immunoglobulins (antibody) which appear in response to the introduction of a micro- or macromolecule) foreign to that body and bind the body-foreign structure initiating its destruction. Such a body-foreign molecule is called an antigen. The antibody is directed against the antigenic determinant or hapten of the antigen e.g. an amino acid sequence, parts of oligosaccharides, polysaccharides, lipopolysaccharides, glycoproteins, lipoproteins, lipoteichoic acids.

The specific antibodies generated in this manner can combine with the antigen which elicited their formation to form an antigen-antibody complex. Antibody molecules have binding sites that are very specific for and complementary to the structural features of the antigen that induced their formation.

This highly specific antigen-antibody recognition and binding has found several applications such as recognition agent, binding agent or carrier agent in various domains such as analytical chemistry, therapeutic treatment, health and beauty care.

EP 479 600, EP 453 097 and EP 450 800 relate to the use of antibodies or fragments thereof for the delivery of active ingredients to a target site. EP 481 701 discloses treatment compositions for topical application containing microcapsules which enclose a beneficial agent at a target location, the microcapsules having an antibody or antibody fragment specific to the target location or a lectin.

WO92/04380 describes reshaped human antibody or reshaped human antibody fragments having specificity for human polymorphic epithelial mucin to be used in the treatment or diagnosis of cancer. The use of Epstein-Barr virus specific polypeptides for the production of antibodies and the diagnostic and treatment of said disease is disclosed in WO94/06470.

Oral compositions comprising antibodies as anti-caries or periodontal diseases treatment have been extensively described in WO95/01155, WO95/00110, WO95/10612, EP 140 498, GB 2 151 923, GB 2 176 400, GB 2 167 299, DE 4324859, US 5 401 723 and EP 280 576.

EP 673 683 and EP 542 309 disclose hair cosmetic compositions containing an antibody to hair or hair extract, obtained from egg yolk or poultry immunised with the hair or hair extract and a polymer emulsion to provide reduced hair damage, softness, moistened feel and smoothness, said composition being adsorbed only onto a specified part of the hair.

Compositions containing antagonists (tyrphostins or antibodies) against epidermal and transforming growth factors, suitable for use in treatment of acne are described in WO95/24896.

The use of antibodies in the overall detergency context has been suggested in Unilever Researchprijs "Molecule zoekt partner" 1992 wherein

modofied antibodies directed to specific stains are proposed to be used in bleaching process.

The production of antibodies by hyperimmunisation of mammals such as a cow with a vaccine derived from *E. coli* bacteria is described in EP 102 831. EP 400 569 discloses a method for preparing vaccine composition for dental caries in nasal drops comprising an antigen produced by integrating a protein antigen-expressing gene into the chromosomal gene of a streptococcus mutants GS-5 strain. WO94/25591 discloses the production of antibodies or functionalised fragments thereof derived from heavy chain immunoglobulins of camelidae.

Detergent compositions include nowadays a complex combination of active ingredients which fulfill certain specific needs : a surfactant system, enzymes providing cleaning and fabric care benefits, bleaching agents, a builder system, suds suppressors, soil-suspending agents, soil-release agents, optical brighteners, softening agents, dispersants, dye transfer inhibition compounds, abrasives, bactericides, perfumes, and their overall performance has indeed improved over the years.

In particular, current laundry detergent formulations generally include detergent enzymes and more specifically proteases.

However, overexposure of the substrate to the proteolytic activity and/or transfer of the proteolytic activity to the post mainwash part of the washing cycle such as rinse, spinning and/or drying steps can lead to unwanted effects. It is well known that the detergent proteolytic enzymes can under certain circumstances lower the tensile strength of the wool constituents of fabrics. It should be noted however, that tensile strength loss of fabric is also an unavoidable result of mechanical action due to use/wearing and may further result from damage by a bleaching component in the laundry process, especially if the fabric is contaminated with metal compounds.

It has now been surprisingly found that the application of antibodies raised against the proteolytic enzyme prevents the occurrence of undesirable residual proteolytic activity. The proteolytic activity can be fully controlled during the cleaning process so that the negative effects due to the overexposure of the substrate to the proteolytic enzyme can now be avoided.

It is therefore an object of the present invention to provide a protease-containing laundry detergent composition delivering excellent cleaning benefits such as stain and/or soil removal, dingy cleaning and fabric care benefits.

The above need has been met by specific laundry detergent compositions comprising a protease and an antibody directed to said proteolytic enzyme.

SUMMARY of the INVENTION

The present invention relates to laundry detergent compositions comprising a protease enzyme and an antibody raised against the proteolytic enzyme in order to provide excellent cleaning performance and fabric care performance.

DETAILED DESCRIPTION of the INVENTION

ANTIBODY

An essential element of the detergent compositions of the present invention is an antibody.

The immunoglobulins are classified into 5 classes, respectively : IgM, IgG, IgA, IgD and IgE. Preferred types of immunoglobulins are IgG and IgA. Secretory IgA which are found into human excreted body fluids such as milk, saliva, respiratory and intestinal fluids are especially designed to survive in said secretions, they have enhanced binding characteristics and are resistant to proteolytic hydrolysis.

The antibody which may be monoclonal or polyclonal or an antibody fragment, may be generated by techniques conventional in the art, for example by using recombinant DNA techniques allowing to produce antibodies variants with new properties : reduced immunogenicity, enhanced affinity, altered size, ... Specific binding may also be used. Preferred for the purpose of the present

invention is a monoclonal antibody, more preferred is a fragment thereof. These fragments may be similarly generated by conventional techniques such as enzymatic digestion by papain or pepsin, or using recombinant DNA techniques. Antibody fragments may also be generated by conventional recombinant DNA techniques. Antibodies and antibodies' fragments may be humanised, such as described in Meded. - Fac. Landbouwkd. Toegepast Biol. Wet. (Univ. Gent) (1995), 60(4a, Forum for Applied Biotechnology, 1995, Part 1), 2057-63.

Heavy and light chains are indeed composed of constant and variable domains. In the organisms producing immunoglobulins in their natural state the constant domains are very important for a number of functions, but for many applications in industrial processes and products their variable domains are sufficient. Consequently many methods have been described to produce antibody fragments.

Antibody fragments which are used may be a Fab, a Fv, a scFv or any other fragment having similar binding properties. Preferred routes to antibodies fragments are through recombinant DNA technology, so that the fragment is expressed by a genetically transformed organism.

Antibodies and antibody fragments produced by recombinant DNA technology do not need to be identical to fragment of antibodies produced in vertebrates, having nevertheless the same binding properties evaluated by their Km, Ki and Kcat. For instance they may include sequences of amino acids and/or glycosylations which differ from those found in antibodies produced in other ways, especially sequences at the ends of fragments. Somewhat analogously, antibody fragments produced through recombinant DNA technology may include extra amino acid sequences at their termini which have no counterpart in antibodies produced in other ways.

A related possibility is that a binding agent for use in this invention is a natural or synthetic polymer which mimics the specific binding activity of a natural antibody's complementary region(s). Such a polymer is for example a polypeptide or a polymer imprinting (Angew. Chem. Int. Ed. Engl. 1995, 34, 1812-1832).

The usual method for the production of antibodies may be adopted in immunising mammals or poultry with the corresponding antigens. As mammals to be immunised, mice, rabbits, goats, sheep, horses, cows, etc. may be used. The antibody (immunoglobulin fraction) may be separated from the antiserum, the milk or the eggs according to the ordinary antibody purification method including salting-out method, Polson extraction, gel-filtration chromatography, ion-exchange chromatography, affinity chromatography and the like, the salting-out method using ammonium sulfate to produce the precipitates, followed by dialysing the precipitates against physiological saline to obtain the purified precipitates as the antibody.

Plants are also capable of synthesising and assembling every kind of antibody molecule and allow a large scale of production of antibodies as described in Tibtech. Dec 1995, Vol 13, pp 522-527; Plant Mol. Biol., 26, pp 1701-1710, 1994 and Biotechnol. proj. 1991, 7, pp 455-461 and in US patent 5, 202,422.

Antibodies can also be produced into microorganisms such as *E. coli* or *S. cerevisiae* via biofermentation process as illustrated in the EP patent 667 394.

Techniques for the production of antibody fragments are well known in the literature : Saiki et al. Science 230 1350-54 (1985); Orlandi et al. PNAS USA 86 3833-7 (1989); WO89/09825; EP 368 684; WO 91/08482 and WO94/25591.

The drawbacks due to prolonged activity of the enzyme can be avoided by an effective control of the enzymatic activity through the introduction of the specifically corresponding antibody. Such antibodies can be either polyclonal - directed to the whole enzyme structure - or monoclonal - directed to specific epitopes of the enzyme activity controlling regions of the enzyme structure. Antibodies raised against specific enzyme can effectively deactivate the enzyme by the antibody-antigen binding in or very near the active site. The formation of such complex leads to the enzyme deactivation and could be explained by the distortion of the 3-dimensional structure and/or steric hindrance at the substrate cleft. The deactivation of the enzyme can also be achieved by the precipitation of the complex antibody-antigen from the washing solution. Due to very high specificity and efficiency of the antibody-antigen interaction, no other detergent active is thereby affected.

The protease-directed antibodies are preferably included into the detergent compositions of the present invention at a level of from 10E-6% to 10E+1% by weight of total composition. In some instances, antibodies raised against specific proteases have the capability of binding other proteases of high structural similarity, providing cross-reactivity. Typically, a molecular ratio of protease-directed antibody to protease will be of 100:1 or lower, preferably of 50:1 or lower. For monoclonal antibodies or fragments thereof, the molecular ratio of protease-directed antibody to protease will be generally of 50:1 or lower, preferably of 20:1 or lower.

The antibodies raised against the protease are released in the wash solution after a lag-period allowing the protease to deliver excellent performance benefits to be achieved by the end of the wash process.

Therefore, the antibodies are preferably incorporated into a release agent in order to control their release timing and rate in the wash solution. The physical form of the antibody-containing release agent is adapted to the physical form of the corresponding detergent or additive.

For granular and powder detergent and cleaning products, the antibodies and release agents can be contained in a granulate. Said antibody granulate can suitably contain various granulation aids, binders, fillers, plasticizers, lubricants, cores and the like. Examples thereof include cellulose (e.g. cellulosic fibers or in microcrystalline form), cellulose derivatives (CMC, MC, HPC, HPMC), gelatin, starch, dextrans, sugars, polyvinylpyrrolidone, PVA, PEG, salts (e.g. sodium sulfate, calcium sulfate), titanium dioxide, talc, clays (kaolin or bentonite) and nonionic surfactants. Other materials of relevance for incorporation in the granulate are described in EP 304 331.

The release agent may be, for example, a coating. Said coating protects said granulates in the wash environment for a certain period of time. The coating will normally be applied to said granulates in an amount in the range of 1% to 50% by weight (calculated on the basis of the weight of the uncoated, dry granulate), preferably in the range of 5 % to 40 % by weight. The amount of coating to be applied to said granulates will depend to a considerable extent on

the nature and composition of the desired coating, and to the kind of protection said coating should offer to said granulates. For example, the thickness of said coating or a multi-layered coating applied onto any of the above granulates may determine the period in which the content of said granulates is released. A possible multi-layered coating may be a coating in which, for example, a fast release coating is applied over a slow release coating.

Also co-granulates can be constructed containing in the outer layer the detergent enzyme and a fast releasing agent and in the inner core, the antibody and a slow releasing agent.

Suitable release coatings are coatings which give rise to release of the contents of antibody-containing granulates under the conditions prevailing during the use thereof. Thus, for example, when a preparation of the invention is to be introduced into a washing liquor containing a washing detergent (normally comprising, e.g. one or more types of surfactants), the coating should be one which ensures the release of the contents of said granulates from the release agent when it is introduced into the washing medium.

Preferred release coatings are coatings which are substantially insoluble in water. Release coatings which are appropriate in washing media may suitably comprise substances selected from the following: cellulose and cellulase derivatives, PVA, PVP, tallow; hydrogenated tallow; partially hydrolyzed tallow; fatty acids and fatty alcohols of natural and synthetic origin; long-chain fatty acid mono-, di- and triesters of glycerol (e.g. glycerol monostearate); ethoxylated fatty alcohols; latexes; hydrocarbons of melting point in the range of 50-80°C; and waxes. Melt-coating agents are a preferred class of fast or slow release coating agents which can be used without dilution with water. Reference may be made to *Controlled Release Systems : Fabrication Technology, Vol. I, CRC Press, 1988*, for further information on slow release coating.

Coatings may suitably further comprise substances such as clays (e.g. kaolin), titanium dioxide, pigments, salts (such as calcium carbonate) and the like. The person skilled in the art will be aware of further coating constituents of relevance in the present invention.

In liquid detergent compositions, the antibody can be incorporated as a dispersion of particles containing in addition to the antibody, a release agent. The antibody can be present in a liquid or solid form. Suitable particles consist of a porous hydrophobic material (e.g., silica with an average pore diameter of 500 Angstrom or higher) containing into the pores a solution of antibodies and a surfactant as described in EP 583 512 of Surutzidis A. et al.

The release agent might be a coating which protects said particles in the wash cycle for a certain period of time. The coating is preferably a hydrophobic coating material such as a hydrophobic liquid polymer. Said polymer can be an organo polysiloxane oil, alternatively a high molecular weight hydrocarbon or water insoluble but water permeable polymeric material such as carboxymethylcellulose, PVA, PVP. The polymer properties are selected to achieve a suitable release profile of the antibody in the wash solution.

THE PROTEASE ENZYME

The proteolytic enzymes are incorporated in the detergent compositions of the present invention at a level of from 0.0001% to 2%, preferably from 0.001% to 0.2%, more preferably from 0.005% to 0.1% pure enzyme by weight of the composition.

The proteolytic enzyme can be of various origin, e.g. produced by mammals, plants, microorganisms. Origin can further be mesophilic or extremophilic (psychrophilic, psychrotrophic, thermophilic, barophilic, alkalophilic, acidophilic, halophilic, etc.). Microorganisms are preferred producers of proteolytic enzymes. Very preferred are serine proteases of bacterial origin. Purified or non-purified forms of these enzymes may be used. Also included by definition, are mutants of native enzymes. Mutants can be obtained e.g. by protein and/or genetic engineering, chemical and/or physical modifications of native enzymes. Common practice as well is the expression of the enzyme via host organism in which the genetic material responsible for the production of the enzyme has been cloned.

Suitable proteases are the subtilisins which are obtained from particular strains of *B. subtilis* and *B. licheniformis* (subtilisin BPN and BPN'). One suitable

protease is obtained from a strain of *Bacillus*, having maximum activity throughout the pH range of 8-12, developed and sold as ESPERASE® by Novo Industries A/S of Denmark, hereinafter "Novo". The preparation of this enzyme and analogous enzymes is described in GB 1,243,784 to Novo. Other suitable proteases include ALCALASE®, DURAZYM® and SAVINASE® from Novo and MAXATASE®, MAXACAL®, PROPERASE® and MAXAPEM® (prot in engineered Maxacal) from Gist-Brocades. Proteolytic enzymes also encompass modified bacterial serine proteases, such as those described in European Patent Application Serial Number 87 303761.8, filed April 28, 1987 (particularly pages 17, 24 and 98), and which is called herein "Protease B", and in European Patent Application 199,404, Venegas, published October 29, 1986, which refers to a modified bacterial serine proteolytic enzyme which is called "Protease A" herein. Suitable is the protease called herein "Protease C", which is a variant of an alkaline serine protease from *Bacillus* in which lysine replaced arginine at position 27, tyrosine replaced valine at position 104, serine replaced asparagine at position 123, and alanine replaced threonine at position 274. Protease C is described in EP 90915958:4, corresponding to WO 91/06637, Published May 16, 1991. Genetically modified variants, particularly of Protease C, are also included herein.

A preferred protease referred to as "Protease D" is a carbonyl hydrolase variant having an amino acid sequence not found in nature, which is derived from a precursor carbonyl hydrolase by substituting a different amino acid for a plurality of amino acid residues at a position in said carbonyl hydrolase equivalent to position +76, preferably also in combination with one or more amino acid residue positions equivalent to those selected from the group consisting of +99, +101, +103, +104, +107, +123, +27, +105, +109, +126, +128, +135, +156, +166, +195, +197, +204, +206, +210, +216, +217, +218, +222, +260, +265, and/or +274 according to the numbering of *Bacillus amyloliquefaciens* subtilisin, as described in WO95/10591 and in the patent application of C. Ghosh, et al, "Bleaching Compositions Comprising Protease Enzymes" having US Serial No. 08/322,677, filed October 13, 1994.

Also suitable for the present invention are proteases described in patent applications EP 251 446 and WO 91/06637, protease BLAP® described in WO91/02792 and their variants described in WO 95/23221.

See also a high pH protease from *Bacillus* sp. NCIMB 40338 described in WO 93/18140 A to Novo. Enzymatic detergents comprising protease, one or more

other enzymes, and a reversible protease inhibitor are described in WO 92/03529 A to Novo. When desired, a protease having decreased adsorption and increased hydrolysis is available as described in WO 95/07791 to Procter & Gamble. A recombinant trypsin-like protease for detergents suitable herein is described in WO 94/25583 to Novo. Other suitable proteases are described in EP 516 200 by Unilever.

The composition of the present invention can also include protease enzymes which are non-naturally-occurring carbonyl hydrolase variants having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. As stated earlier, the protease enzymes are designed to have trypsin-like specificity and preferably also be bleach stable. The precursor carbonyl hydrolase may be a naturally-occurring carbonyl hydrolase or recombinant hydrolase. Specifically, such carbonyl hydrolase variants have an amino acid sequence not found in nature, which is derived by replacement of a plurality of amino acid residues of a precursor carbonyl hydrolase with different amino acids. The plurality of amino acid residues of the precursor enzyme correspond to position +210 in combination with one or more of the following residues: +33, +62, +67, +76, +100, +101, +103, +104, +107, +128, +129, +130, +132, +135, +156, +158, +164, +166, +167, +170, +209, +215, +217, +218, and +222, where the numbered position corresponds to naturally-occurring subtilisin from *Bacillus amyloliquefaciens* or to equivalent amino acid residues in other carbonyl hydrolases or subtilisins, such as *Bacillus lentinus* subtilisin.

The carbonyl hydrolase variants which are protease enzymes useful in the present invention compositions comprise replacement of amino acid residue +210 in combination with one or more additional modifications. While any combination of the above listed amino acid substitutions may be employed, the preferred variant protease enzymes useful for the present invention comprise the substitution, deletion or insertion of amino acid residues in the following combinations: 210/156; 210/166; 210/76; 210/103; 210/104; 210/217; 210/156/166; 210/156/217; 210/166/217; 210/76/156; 210/76/166; 210/76/217; 210/76/156/166; 210/76/156/217; 210/76/166/217; 210/76/103/156; 210/76/103/166; 210/76/103/217; 210/76/104/156; 210/76/104/166; 210/76/104/217; 210/76/103/104/156; 210/76/103/104/166; 210/76/103/104/217;

210/76/103/104/156/166; 210/76/103/104/156/217; 210/76/103/104/166/217 and/or 210/76/103/104/156/166/217; 210/76/103/104/166/222; 210/67/76/103/104/166/222; 210/67/76/103/104/166/218/222. Most preferably the variant enzymes useful for the present invention comprise the substitution, deletion or insertion of an amino acid residue in the following combination of residues: 210/156; 210/166; 210/217; 210/156/166; 210/156/217; 210/166/217; 210/76/156/166; 210/76/103/156/166 and 210/76/103/104/156/166 of *B. lenth* subtilisin with 210/76/103/104/156/166 being the most preferred.

Variant DNA sequences encoding such carbonyl hydrolase or subtilisin variants are derived from a precursor DNA sequence which encodes a naturally-occurring or recombinant precursor enzyme. The variant DNA sequences are derived by modifying the precursor DNA sequence to encode the substitution of one or more specific amino acid residues encoded by the precursor DNA sequence corresponding to positions +210, +33, +62, +67, +76, +100, +101, +103, +104, +107, +128, +129, +130, +132, +135, +156, +158, +164, +166, +167, +170, +209, +215, +217, +218, and +222 in *Bacillus lenth* or any combination thereof. Although the amino acid residues identified for modification herein are identified according to the numbering applicable to *B. amyloliquefaciens* (which has become the conventional method for identifying residue positions in all subtilisins), the preferred precursor DNA sequence useful for the present invention is the DNA sequence of *Bacillus lenth*. These recombinant DNA sequences encode carbonyl hydrolase variants having a novel amino acid sequence and, in general, at least one property which is substantially different from the same property of the enzyme encoded by the precursor carbonyl hydrolase DNA sequence. Such properties include proteolytic activity, substrate specificity, stability, altered pH profile and/or enhanced performance characteristics.

The protease enzymes useful herein encompass the substitution of any of the nineteen naturally occurring L-amino acids at the designated amino acid residue positions. Such substitutions can be made in any precursor subtilisin (procaryotic, eucaryotic, mammalian, etc.). Throughout this application reference is made to various amino acids by way of common one- and three-letter codes. Such codes are identified in Dale, M.W. (1989), Molecular Genetics of Bacteria, John Wiley & Sons, Ltd., Appendix B.

Pref rably, the substitution to be mad at each of the identified amino acid residue positions include but are not limited to substitutions at position +210

including I, V, L, and A, substitutions at positions +33, +62, +76, +100, +101, +103, +104, +107, +128, +129, +130, +132, +135, +156, +158, +164, +166, +167, +170, +209, +215, +217, and +218 of D or E, substitutions at position 76 including D, H, E, G, F, K, P and N; substitutions at position 103 including Q, T, D, E, Y, K, G, R and S; and substitutions at position 104 including S, Y, I, L, M, A, W, D, T, G and V; and substitutions at position 222 including S, C, A. The specifically preferred amino acid(s) to be substituted at each such position are designated below in Table I. Although specific amino acids are shown in Table I, it should be understood that any amino acid may be substituted at the identified residues.

Table I

<u>Amino Acid residue</u>	<u>Preferred Amino Acid to be Substituted/Inserted</u>
+ 210	I, V, L, A
+33, +62, +100, +101, +107	D, E
+128, +129, +130, +135	
+156, +158, +164, +166	
+167, +170, +209, +215	
+217 and +218	
+ 76	D, H
+ 103	A, Q, T, D, E, Y, K, G, R
+ 104	I, Y, S, L, A, T, G
+ 222	S, C, A

A comparison of the preferred amino acid residues identified herein for substitution versus the preferred substitution for each such position is provided in Table II.

Table II

	+210	+156	+166	+217	+76	+103	+104
<i>B. amyloliquefaciens</i> (wild-type)	P	E	G	Y	N	Q	Y

<i>B. lenthus</i> (wild-type)	P	S	S	L	N	S	V
Most Preferred	I	E/D	E/D	E/D	D	A	I/Y
Substitution							

Detergent components

The detergent compositions of the invention may also contain additional detergent components. The precise nature of these additional components, and levels of incorporation thereof will depend on the physical form of the composition, and the nature of the cleaning operation for which it is to be used.

The detergent compositions according to the invention can be liquid, paste, gels, bars, tablets, powder or granular forms. Granular compositions can also be in "compact" form, the liquid compositions can also be in a "concentrated" form.

The compositions of the invention may be formulated as hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the soaking and/or pretreatment of stained fabrics, rinse added fabric softener compositions. Such compositions can provide fabric cleaning, stain removal, whiteness maintenance, softening, color appearance and dye transfer inhibition.

When formulated as compositions suitable for use in a laundry machine washing method, the compositions of the invention preferably contain both a surfactant and a builder compound and additionally one or more detergent components preferably selected from organic polymeric compounds, bleaching agents, additional enzymes, suds suppressors, dispersants, lime-soap dispersants, soil suspension and anti-redeposition agents and corrosion inhibitors. Laundry compositions can also contain softening agents, as additional detergent components.

The compositions of the invention can also be used as detergent additive products comprising a protease-directed antibody and will be added to a

conventional detergent protease-containing compositions. The detergent additives can also comprise the protease and the protease-directed antibody. Such additives products are intended to supplement or boost the performance of conventional detergent compositions and preferably comprise up to 50% antibodies by weight of total composition.

If needed the density of the laundry detergent compositions herein ranges from 400 to 1200 g/litre, preferably 600 to 950 g/litre of composition measured at 20°C.

The "compact" form of the compositions herein is best reflected by density and, in terms of composition, by the amount of inorganic filler salt; inorganic filler salts are conventional ingredients of detergent compositions in powder form; in conventional detergent compositions, the filler salts are present in substantial amounts, typically 17-35% by weight of the total composition.

In the compact compositions, the filler salt is present in amounts not exceeding 15% of the total composition, preferably not exceeding 10%, most preferably not exceeding 5% by weight of the composition.

The inorganic filler salts, such as meant in the present compositions are selected from the alkali and alkaline-earth-metal salts of sulphates and chlorides.

A preferred filler salt is sodium sulphate.

Liquid detergent compositions according to the present invention can also be in a "concentrated form", in such case, the liquid detergent compositions according the present invention will contain a lower amount of water, compared to conventional liquid detergents.

Typically the water content of the concentrated liquid detergent is preferably less than 40%, more preferably less than 30%, most preferably less than 20% by weight of the detergent composition.

Surfactant system

The detergent compositions according to the present invention comprise a surfactant system wherein the surfactant can be selected from nonionic and/or anionic and/or cationic and/or ampholytic and/or zwitterionic and/or semi-polar surfactants.

The surfactant is typically present at a level of from 0.1% to 60% by weight. More preferred levels of incorporation are 1% to 35% by weight, most preferably from 1% to 30% by weight of detergent compositions in accord with the invention.

The surfactant is preferably formulated to be compatible with enzyme components present in the composition. In liquid or gel compositions the surfactant is most preferably formulated such that it promotes, or at least does not degrade, the stability of any enzyme in these compositions.

Preferred surfactant systems to be used according to the present invention comprise as a surfactant one or more of the nonionic and/or anionic surfactants described herein.

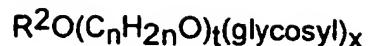
Polyethylene, polypropylene, and polybutylene oxide condensates of alkyl phenols are suitable for use as the nonionic surfactant of the surfactant systems of the present invention, with the polyethylene oxide condensates being preferred. These compounds include the condensation products of alkyl phenols having an alkyl group containing from about 6 to about 14 carbon atoms, preferably from about 8 to about 14 carbon atoms, in either a straight-chain or branched-chain configuration with the alkylene oxide. In a preferred embodiment, the ethylene oxide is present in an amount equal to from about 2 to about 25 moles, more preferably from about 3 to about 15 moles, of ethylene oxide per mole of alkyl phenol. Commercially available nonionic surfactants of this type include IgepalTM CO-630, marketed by the GAF Corporation; and TritonTM X-45, X-114, X-100 and X-102, all marketed by the Rohm & Haas Company. These surfactants are commonly referred to as alkylphenol alkoxylates (e.g., alkyl phenol ethoxylates).

The condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethylene oxide are suitable for use as the nonionic surfactant of the nonionic surfactant systems of the present invention. The alkyl chain of the aliphatic alcohol can either be straight or branched, primary or secondary, and generally contains from about 8 to about 22 carbon atoms. Preferred are the condensation products of alcohols having an alkyl group containing from about 8 to about 20 carbon atoms, more preferably from about

10 to about 18 carbon atoms, with from about 2 to about 10 moles of ethylene oxide per mole of alcohol. About 2 to about 7 moles of ethylene oxide and most preferably from 2 to 5 moles of ethylene oxide per mole of alcohol are present in said condensation products. Examples of commercially available nonionic surfactants of this type include TergitolTM 15-S-9 (the condensation product of C₁₁-C₁₅ linear alcohol with 9 moles ethylene oxide), TergitolTM 24-L-6 NMW (the condensation product of C₁₂-C₁₄ primary alcohol with 6 moles ethylene oxide with a narrow molecular weight distribution), both marketed by Union Carbide Corporation; NeodolTM 45-9 (the condensation product of C₁₄-C₁₅ linear alcohol with 9 moles of ethylene oxide), NeodolTM 23-3 (the condensation product of C₁₂-C₁₃ linear alcohol with 3.0 moles of ethylene oxide), NeodolTM 45-7 (the condensation product of C₁₄-C₁₅ linear alcohol with 7 moles of ethylene oxide), NeodolTM 45-5 (the condensation product of C₁₄-C₁₅ linear alcohol with 5 moles of ethylene oxide) marketed by Shell Chemical Company, KyroTM EOB (the condensation product of C₁₃-C₁₅ alcohol with 9 moles ethylene oxide), marketed by The Procter & Gamble Company, and Genapol LA O3O or O5O (the condensation product of C₁₂-C₁₄ alcohol with 3 or 5 moles of ethylene oxide) marketed by Hoechst. Preferred range of HLB in these products is from 8-11 and most preferred from 8-10.

Also useful as the nonionic surfactant of the surfactant systems of the present invention are the alkylpolysaccharides disclosed in U.S. Patent 4,565,647, Llenado, issued January 21, 1986, having a hydrophobic group containing from about 6 to about 30 carbon atoms, preferably from about 10 to about 16 carbon atoms and a polysaccharide, e.g. a polyglycoside, hydrophilic group containing from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7 saccharide units. Any reducing saccharide containing 5 or 6 carbon atoms can be used, e.g., glucose, galactose and galactosyl moieties can be substituted for the glucosyl moieties (optionally the hydrophobic group is attached at the 2-, 3-, 4-, etc. positions thus giving a glucose or galactose as opposed to a glucoside or galactoside). The intersaccharide bonds can be, e.g., between the one position of the additional saccharide units and the 2-, 3-, 4-, and/or 6- positions on the preceding saccharide units.

The preferred alkylpolyglycosides have the formula



wherein R^2 is selected from the group consisting of alkyl, alkylphenyl, hydroxyalkyl, hydroxyalkylphenyl, and mixtures thereof in which the alkyl groups contain from about 10 to about 18, preferably from about 12 to about 14, carbon atoms; n is 2 or 3, preferably 2; t is from 0 to about 10, preferably 0; and x is from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7. The glycosyl is preferably derived from glucose. To prepare these compounds, the alcohol or alkylpolyethoxy alcohol is formed first and then reacted with glucose, or a source of glucose, to form the glucoside (attachment at the 1-position). The additional glycosyl units can then be attached between their 1-position and the preceding glycosyl units 2-, 3-, 4- and/or 6-position, preferably predominately the 2-position.

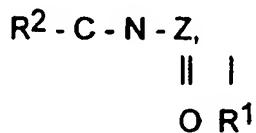
The condensation products of ethylene oxide with a hydrophobic base form d by the condensation of propylene oxide with propylene glycol are also suitable for use as the additional nonionic surfactant systems of the present invention. The hydrophobic portion of these compounds will preferably have a molecular weight of from about 1500 to about 1800 and will exhibit water insolubility. The addition of polyoxyethylene moieties to this hydrophobic portion tends to increase the water solubility of the molecule as a whole, and the liquid character of the product is retained up to the point where the polyoxyethylene content is about 50% of the total weight of the condensation product, which corresponds to condensation with up to about 40 moles of ethylene oxide. Examples of compounds of this type include certain of the commercially-available PlurafacTM LF404 and PluronicTM surfactants, marketed by BASF.

Also suitable for use as the nonionic surfactant of the nonionic surfactant system of the present invention, are the condensation products of ethylene oxide with the product resulting from the reaction of propylene oxide and ethylenediamine. The hydrophobic moiety of these products consists of the reaction product of ethylenediamine and excess propylene oxide, and generally has a molecular weight of from about 2500 to about 3000. This hydrophobic moiety is condensed with ethylene oxide to the extent that the condensation product contains from about 40% to about 80% by weight of polyoxyethylene and has a molecular weight of from about 5,000 to about 11,000. Examples of this

type of nonionic surfactant include certain of the commercially available TetronicTM compounds, marketed by BASF.

Preferred for use as the nonionic surfactant of the surfactant systems of the present invention are polyethylene oxide condensates of alkyl phenols, condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethylene oxide, alkylpolysaccharides, and mixtures thereof. Most preferred are C₈-C₁₄ alkyl phenol ethoxylates having from 3 to 15 ethoxy groups and C₈-C₁₈ alcohol ethoxylates (preferably C₁₀ avg.) having from 2 to 10 ethoxy groups, and mixtures thereof.

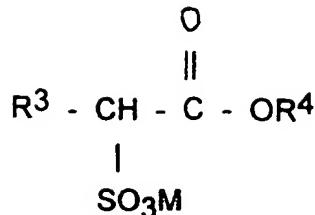
Highly preferred nonionic surfactants are polyhydroxy fatty acid amid surfactants of the formula.



wherein R¹ is H, or R¹ is C₁₋₄ hydrocarbyl, 2-hydroxy ethyl, 2-hydroxy propyl or a mixture thereof, R² is C₅₋₃₁ hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably, R¹ is methyl, R² is a straight C₁₁₋₁₅ alkyl or C₁₆₋₁₈ alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose, lactose, in a reductive amination reaction.

Suitable anionic surfactants to be used are linear alkyl benzene sulfonate, alkyl ester sulfonate surfactants including linear esters of C₈-C₂₀ carboxylic acids (i.e., fatty acids) which are sulfonated with gaseous SO₃ according to "The Journal of the American Oil Chemists Society", 52 (1975), pp. 323-329. Suitable starting materials would include natural fatty substances as derived from tallow, palm oil, etc.

The preferred alkyl ester sulfonate surfactant, especially for laundry applications, comprise alkyl ester sulfonate surfactants of the structural formula :



wherein R^3 is a C₈-C₂₀ hydrocarbyl, preferably an alkyl, or combination thereof, R^4 is a C₁-C₆ hydrocarbyl, preferably an alkyl, or combination thereof, and M is a cation which forms a water soluble salt with the alkyl ester sulfonate. Suitable salt-forming cations include metals such as sodium, potassium, and lithium, and substituted or unsubstituted ammonium cations, such as monoethanolamine, diethanolamine, and triethanolamine. Preferably, R^3 is C₁₀-C₁₆ alkyl, and R^4 is methyl, ethyl or isopropyl. Especially preferred are the methyl ester sulfonates wherein R^3 is C₁₀-C₁₆ alkyl.

Other suitable anionic surfactants include the alkyl sulfate surfactants which are water soluble salts or acids of the formula ROSO_3M wherein R preferably is a C₁₀-C₂₄ hydrocarbyl, preferably an alkyl or hydroxyalkyl having a C₁₀-C₂₀ alkyl component, more preferably a C₁₂-C₁₈ alkyl or hydroxyalkyl, and M is H or a cation, e.g., an alkali metal cation (e.g. sodium, potassium, lithium), or ammonium or substituted ammonium (e.g. methyl-, dimethyl-, and trimethyl ammonium cations and quaternary ammonium cations such as tetramethylammonium and dimethyl piperidinium cations and quaternary ammonium cations derived from alkylamines such as ethylamine, diethylamine, triethylamine, and mixtures thereof, and the like). Typically, alkyl chains of C₁₂-C₁₆ are preferred for lower wash temperatures (e.g. below about 50°C) and C₁₆-C₁₈ alkyl chains are preferred for higher wash temperatures (e.g. above about 50°C).

Other anionic surfactants useful for detergents purposes can also be included in the detergent compositions of the present invention. These can include salts (including, for example, sodium, potassium, ammonium, and substituted ammonium salts such as mono-, di- and triethanolamine salts) of soap, C₈-C₂₂ primary or secondary alkanesulfonates, C₈-C₂₄ olefinsulfonates, sulfonated polycarboxylic acids prepared by sulfonation of the pyrolyzed product of alkali metal citrates, e.g., as described in British patent specification No. 1,082,179, C₈-C₂₄ alkylpolyglycolethersulfates (containing up to 10 moles of ethylene oxide); alkyl glycerol sulfonates, fatty acyl glycerol sulfonates, fatty oleyl

glycerol sulfates, alkyl phenol ethylene oxide ether sulfates, paraffin sulfonates, alkyl phosphates, isethionates such as the acyl isethionates, N-acyl taurates, alkyl succinamates and sulfosuccinates, monoesters of sulfosuccinates (especially saturated and unsaturated C₁₂-C₁₈ monoesters) and diesters of sulfosuccinates (especially saturated and unsaturated C₆-C₁₂ diesters), acyl sarcosinates, sulfates of alkylpolysaccharides such as the sulfates of alkylpolyglucoside (the nonionic nonsulfated compounds being described below), branched primary alkyl sulfates, and alkyl polyethoxy carboxylates such as those of the formula RO(CH₂CH₂O)_k-CH₂COO-M⁺ wherein R is a C₈-C₂₂ alkyl, k is an integer from 1 to 10, and M is a soluble salt-forming cation. Resin acids and hydrogenated resin acids are also suitable, such as rosin, hydrogenated rosin, and resin acids and hydrogenated resin acids present in or derived from tall oil.

Further examples are described in "Surface Active Agents and Detergents" (Vol. I and II by Schwartz, Perry and Berch). A variety of such surfactants are also generally disclosed in U.S. Patent 3,929,678, issued December 30, 1975 to Laughlin, et al. at Column 23, line 58 through Column 29, line 23 (herein incorporated by reference).

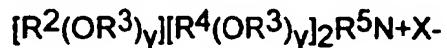
When included therein, the laundry detergent compositions of the present invention typically comprise from about 1% to about 40%, preferably from about 3% to about 20% by weight of such anionic surfactants.

Highly preferred anionic surfactants include alkyl alkoxyolated sulfate surfactants hereof are water soluble salts or acids of the formula RO(A)_mSO₃M wherein R is an unsubstituted C₁₀-C₂₄ alkyl or hydroxyalkyl group having a C₁₀-C₂₄ alkyl component, preferably a C₁₂-C₂₀ alkyl or hydroxyalkyl, more preferably C₁₂-C₁₈ alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater than zero, typically between about 0.5 and about 6, more preferably between about 0.5 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxylated sulfates as well as alkyl propoxylated sulfates are contemplated herein. Specific examples of substituted ammonium cations include methyl-, dimethyl, trimethyl-ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperidinium cations and those derived from alkylamines such as ethylamine, diethylamine, triethylamine, mixtures thereof, and the like. Exemplary

surfactants are C₁₂-C₁₈ alkyl polyethoxylate (1.0) sulfate (C₁₂-C₁₈E(1.0)M), C₁₂-C₁₈ alkyl polyethoxylate (2.25) sulfate (C₁₂-C₁₈E(2.25)M), C₁₂-C₁₈ alkyl polyethoxylate (3.0) sulfate (C₁₂-C₁₈E(3.0)M), and C₁₂-C₁₈ alkyl polyethoxylate (4.0) sulfate (C₁₂-C₁₈E(4.0)M), wherein M is conveniently selected from sodium and potassium.

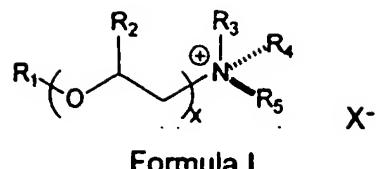
The detergent compositions of the present invention may also contain cationic, ampholytic, zwitterionic, and semi-polar surfactants, as well as the nonionic and/or anionic surfactants other than those already described herein.

Cationic detergents suitable for use in the detergent compositions of the present invention are those having one long-chain hydrocarbyl group. Examples of such cationic surfactants include the ammonium surfactants such as alkyltrimethylammonium halogenides, and those surfactants having the formula:

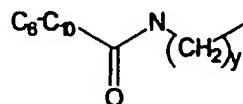


wherein R² is an alkyl or alkyl benzyl group having from about 8 to about 18 carbon atoms in the alkyl chain, each R³ is selected from the group consisting of -CH₂CH₂-, -CH₂CH(CH₃)-, -CH₂CH(CH₂OH)-, -CH₂CH₂CH₂-, and mixtures thereof; each R⁴ is selected from the group consisting of C₁-C₄ alkyl, C₁-C₄ hydroxyalkyl, benzyl ring structures formed by joining the two R⁴ groups, -CH₂CHOH-CHOHCOR⁶CHOHCH₂OH wherein R⁶ is any hexose or hexose polymer having a molecular weight less than about 1000, and hydrogen when y is not 0; R⁵ is the same as R⁴ or is an alkyl chain wherein the total number of carbon atoms of R² plus R⁵ is not more than about 18; each y is from 0 to about 10 and the sum of the y values is from 0 to about 15; and X is any compatible anion.

Quaternary ammonium surfactant suitable for the present invention has the formula (I):



whereby R1 is a short chainlength alkyl (C6-C10) or alkylamidoalkyl of the formula (II) :



Formula II

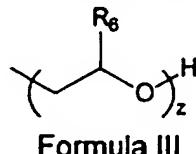
y is 2-4, preferably 3.

whereby R2 is H or a C1-C3 alkyl,

whereby x is 0-4, preferably 0-2, most preferably 0,

whereby R3, R4 and R5 are either the same or different and can be either a short chain alkyl (C1-C3) or alkoxylated alkyl of the formula III,

whereby X- is a counterion, preferably a halide, e.g. chloride or methylsulfate.



R6 is C1-C4 and z is 1 or 2.

Preferred quat ammonium surfactants are those as defined in formula I whereby

R1 is C8, C10 or mixtures thereof, x=0,

R3, R4 = CH₃ and R5 = CH₂CH₂OH.

Highly preferred cationic surfactants are the water-soluble quaternary ammonium compounds useful in the present composition having the formula :



wherein R₁ is C₈-C₁₆ alkyl, each of R₂, R₃ and R₄ is independently C₁-C₄ alkyl, C₁-C₄ hydroxy alkyl, benzyl, and -(C₂H₄O)_xH where x has a value from 2 to 5, and X is an anion. Not more than one of R₂, R₃ or R₄ should be benzyl.

The preferred alkyl chain length for R₁ is C₁₂-C₁₅ particularly where the alkyl group is a mixture of chain lengths derived from coconut or palm kernel fat or is derived synthetically by olefin build up or OXO alcohols synthesis. Preferred groups for R₂R₃ and R₄ are methyl and hydroxyethyl groups and the anion X may be selected from halide, methosulphate, acetate and phosphate ions.

Examples of suitable quaternary ammonium compounds of formulae (i) for use herein are :

coconut trimethyl ammonium chloride or bromide;
coconut methyl dihydroxyethyl ammonium chloride or bromide;
decyl triethyl ammonium chloride;
decyl dimethyl hydroxyethyl ammonium chloride or bromide;
C₁₂-15 dimethyl hydroxyethyl ammonium chloride or bromide;
coconut dimethyl hydroxyethyl ammonium chloride or bromide;
myristyl trimethyl ammonium methyl sulphate;
lauryl dimethyl benzyl ammonium chloride or bromide;
lauryl dimethyl (ethenoxy)₄ ammonium chloride or bromide;
choline esters (compounds of formula (i) wherein R₁ is
CH₂-CH₂-O-C-C₁₂-14 alkyl and R₂R₃R₄ are methyl).

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di-alkyl imidazolines [compounds of formula (i)].

Other cationic surfactants useful herein are also described in U.S. Patent 4,228,044, Cambre, issued October 14, 1980 and in European Patent Application EP 000,224.

When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 25%, preferably from about 1% to about 8% by weight of such cationic surfactants.

Ampholytic surfactants are also suitable for use in the detergent compositions of the present invention. These surfactants can be broadly described as aliphatic derivatives of secondary or tertiary amines, or aliphatic derivatives of heterocyclic

secondary and tertiary amines in which the aliphatic radical can be straight- or branched-chain. One of the aliphatic substituents contains at least about 8 carbon atoms, typically from about 8 to about 18 carbon atoms, and at least one contains an anionic water-solubilizing group, e.g. carboxy, sulfonate, sulfate. See U.S. Patent No. 3,929,678 to Laughlin et al., issued December 30, 1975 at column 19, lines 18-35, for examples of amphotolytic surfactants.

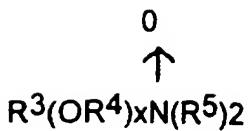
When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such amphotolytic surfactants.

Zwitterionic surfactants are also suitable for use in detergent compositions. These surfactants can be broadly described as derivatives of secondary and tertiary amines, derivatives of heterocyclic secondary and tertiary amines, or derivatives of quaternary ammonium, quaternary phosphonium or tertiary sulfonium compounds. See U.S. Patent No. 3,929,678 to Laughlin et al., issued December 30, 1975 at column 19, line 38 through column 22, line 48, for examples of zwitterionic surfactants.

When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such zwitterionic surfactants.

Semi-polar nonionic surfactants are a special category of nonionic surfactants which include water-soluble amine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; water-soluble phosphine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; and water-soluble sulfoxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and a moiety selected from the group consisting of alkyl and hydroxyalkyl moieties of from about 1 to about 3 carbon atoms.

Semi-polar nonionic detergent surfactants include the amine oxide surfactants having the formula



wherein R^3 is an alkyl, hydroxyalkyl, or alkyl phenyl group or mixtures thereof containing from about 8 to about 22 carbon atoms; R^4 is an alkylene or hydroxyalkylene group containing from about 2 to about 3 carbon atoms or mixtures thereof; x is from 0 to about 3; and each R^5 is an alkyl or hydroxyalkyl group containing from about 1 to about 3 carbon atoms or a polyethylene oxide group containing from about 1 to about 3 ethylene oxide groups. The R^5 groups can be attached to each other, e.g., through an oxygen or nitrogen atom, to form a ring structure.

These amine oxide surfactants in particular include C₁₀-C₁₈ alkyl dimethyl amine oxides and C₈-C₁₂ alkoxy ethyl dihydroxy ethyl amine oxides.

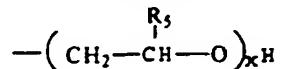
When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such semi-polar nonionic surfactants.

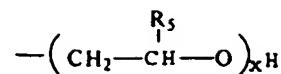
The detergent composition of the present invention may further comprise a cosurfactant selected from the group of primary or tertiary amines.

Suitable primary amines for use herein include amines according to the formula R_1NH_2 wherein R_1 is a C₆-C₁₂, preferably C₆-C₁₀ alkyl chain or $\text{R}_4\text{X}(\text{CH}_2)_n$. X is -O-, -C(O)NH- or -NH-, R_4 is a C₆-C₁₂ alkyl chain n is between 1 to 5, preferably 3. R_1 alkyl chains may be straight or branched and may be interrupted with up to 12, preferably less than 5 ethylene oxide moieties.

Preferred amines according to the formula herein above are n-alkyl amines. Suitable amines for use herein may be selected from 1-hexylamine, 1-octylamine, 1-decylamine and laurylamine. Other preferred primary amines include C₈-C₁₀ oxypropylamine, octyloxypropylamine, 2-ethylhexyl-oxypropylamine, lauryl amido propylamine and amido propylamine.

Suitable tertiary amines for use herein include tertiary amines having the formula $\text{R}_1\text{R}_2\text{R}_3\text{N}$ where R_1 and R_2 are C₁-C₈ alkyl chains or

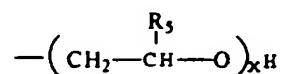




R_3 is either a C₆-C₁₂, preferably C₆-C₁₀ alkyl chain, or R_3 is $\text{R}_4\text{X}(\text{CH}_2)_n$, whereby X is -O-, -C(O)NH- or -NH- R_4 is a C₄-C₁₂, n is between 1 to 5, preferably 2-3. R_5 is H or C₁-C₂ alkyl and x is between 1 to 6.

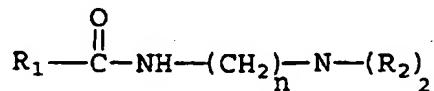
R_3 and R_4 may be linear or branched ; R_3 alkyl chains may be interrupted with up to 12, preferably less than 5, ethylene oxide moieties.

Preferred tertiary amines are $\text{R}_1\text{R}_2\text{R}_3\text{N}$ where R_1 is a C₆-C₁₂ alkyl chain, R_2 and R_3 are C₁-C₃ alkyl or



where R_5 is H or CH₃ and x = 1-2.

Also preferred are the amidoamines of the formula:



wherein R_1 is C₆-C₁₂ alkyl; n is 2-4, preferably n is 3; R_2 and R_3 is C₁-C₄

Most preferred amines of the present invention include 1-octylamine, 1-hexylamine, 1-decylamine, 1-dodecylamine, C8-10oxypropylamine, N coco 1-3diaminopropane, coconutalkyldimethylamine, lauryldimethylamine, lauryl bis(hydroxyethyl)amine, coco bis(hydroxyethyl)amine, lauryl amine 2 moles propoxylated, octyl amine 2 moles propoxylated, lauryl amidopropyldimethylamine, C8-10 amidopropyldimethylamine and C10 amidopropyldimethylamine.

The most preferred amines for use in the compositions herein are 1-hexylamine, 1-octylamine, 1-decylamine, 1-dodecylamine. Especially desirable are n-

dodecyldimethylamine and bishydroxy thylcoconutalkylamine and oleylamine 7
tim s thoxylated, lauryl amido propylamin and cocoamido propylamine.

Conventional detergent enzymes

The detergent compositions can further comprise one or more enzymes which provide detergent performance and/or fabric care benefits.

Said enzymes include enzymes selected from hemicellulases, peroxidases, gluco-amylases, amylases, xylanases, lipases, phospholipase, esterases, cutinases, pectinases, keratanases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, β -glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase or mixtures thereof.

A preferred combination is a detergent composition having cocktail of conventional applicable enzymes like protease, amylase, lipase, cutinase and/or cellulase in conjunction with one or more plant cell wall degrading enzymes.

The cellulases usable in the present invention include both bacterial or fungal cellulase. Preferably, they will have a pH optimum of between 5 and 9.5. Suitable cellulases are disclosed in U.S. Patent 4,435,307, Barbesgaard et al, which discloses fungal cellulase produced from *Humicola insolens*. Suitable cellulases are also disclosed in GB-A-2.075.028; GB-A-2.095.275 and DE-OS-2.247.832.

Examples of such cellulases are cellulases produced by a strain of *Humicola insolens* (*Humicola grisea* var. *thermoidea*), particularly the *Humicola* strain DSM 1800.

Other suitable cellulases are cellulases originated from *Humicola insolens* having a molecular weight of about 50kDa, an isoelectric point of 5.5 and containing 415 amino acids and a 43kD endoglucanase derived from *Humicola insolens*, DSM 1800, exhibiting cellulase activity; a preferred endoglucanase component has the amino acid sequence disclosed in PCT Patent Application No. WO 91/17243. Also suitable cellulases are the EGIII cellulases from *Trichoderma longibrachiatum* described in WO94/21801, Genencor, published September 29,

1994. Especially suitable cellulases are the cellulases having color care benefits. Examples of such cellulases are cellulases described in European patent application No. 91202879.2, filed November 6, 1991 (Novo).

Peroxidase enzymes are used in combination with oxygen sources, e.g. percarbonate, perborate, persulfate, hydrogen peroxide, etc. They are used for "solution bleaching", i.e. to prevent transfer of dyes or pigments removed from substrates during wash operations to other substrates in the wash solution. Peroxidase enzymes are known in the art, and include, for example, horseradish peroxidase, ligninase, and haloperoxidase such as chloro- and bromoperoxidase.

Peroxidase-containing detergent compositions are disclosed, for example, in PCT International Application WO 89/099813 and in European Patent application EP No. 91202882.6, filed on November 6, 1991.

Other suitable oxidase enzymes is the laccase enzyme using hydrogen peroxide, oxygen as primary substrates.

Said cellulases and/or peroxidases are normally incorporated in the detergent composition at levels from 0.0001% to 2% of active enzyme by weight of the detergent composition.

Other preferred enzymes that can be included in the detergent compositions of the present invention include lipases. Suitable lipase enzymes for detergent usage include those produced by microorganisms of the *Pseudomonas* group, such as *Pseudomonas stutzeri* ATCC 19.154, as disclosed in British Patent 1,372,034. Suitable lipases include those which show a positive immunological cross-reaction with the antibody of the lipase, produced by the microorganism *Pseudomonas fluorescent* IAM 1057. This lipase is available from Amano Pharmaceutical Co. Ltd., Nagoya, Japan, under the trade name Lipase P "Amano," hereinafter referred to as "Amano-P". Other suitable commercial lipases include Amano-CES, lipases ex *Chromobacter viscosum*, e.g. *Chromobacter viscosum* var. *lipolyticum* NRRLB 3673 from Toyo Jozo Co., Tagata, Japan; *Chromobacter viscosum* lipases from U.S. Biochemical Corp., U.S.A. and Disoynth Co., The Netherlands, and lipases ex *Pseudomonas gladioli*. Especially suitable lipases are lipases such as M1 Lipase^R and Lipomax^R (Gist-Brocades) and Lipolase^R and Lipolase Ultra^R(Novo) which have

found to be very effective when used in combination with the compositions of the present invention.

Also suitable are cutinases [EC 3.1.1.50] which can be considered as a special kind of lipase, namely lipases which do not require interfacial activation. Addition of cutinases to detergent compositions have been described in e.g. WO-A-88/09367 (Genencor).

The lipases and/or cutinases are normally incorporated in the detergent composition at levels from 0.0001% to 2% of active enzyme by weight of the detergent composition.

Amylases (α and/or β) can be included for removal of carbohydrate-based stains. WO94/02597, Novo Nordisk A/S published February 03, 1994, describes detergent compositions which incorporate mutant amylases. See also WO94/18314, Genencor, published August 18, 1994 and WO95/10603, Novo Nordisk A/S, published April 20, 1995. Other amylases known for use in detergent compositions include both α - and β -amylases. α -Amylases are known in the art and include those disclosed in US Pat. no. 5,003,257; EP 252,666; WO/91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent specification no. 1,296,839 (Novo). Other suitable amylase are stability-enhanced amylases including Purafact Ox Am^R described in WO 94/18314, published August 18, 1994; WO96/05295, Genencor, published February 22, 1996 and amylase variants having additional modification in the immediate parent available from Novo Nordisk A/S, disclosed in WO 95/10603, published April 95.

Examples of commercial α -amylases products are Termamyl[®], Ban[®], Fungamyl[®] and Duramyl[®], all available from Novo Nordisk A/S Denmark. WO95/26397 describes other suitable amylases : α -amylases characterised by having a specific activity at least 25% higher than the specific activity of Termamyl[®] at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by the Phadebas[®] α -amylase activity assay. Other amylolytic enzymes with improved properties with respect to the activity level and the combination of thermostability and a higher activity level are described in WO95/35382.

The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Said enzymes are normally incorporated in the detergent composition at levels from 0.0001% to 2% of active

enzyme by weight of the detergent composition. The enzymes can be added as separate single ingredients (prills, granulates, stabilized liquids, etc., containing one enzyme) or as mixtures of two or more enzymes (e.g. cogranulates).

Other suitable detergent ingredients that can be added are enzyme oxidation scavengers which are described in Co-pending European Patent application 92870018.6 filed on January 31, 1992. Examples of such enzyme oxidation scavengers are ethoxylated tetraethylene polyamines.

A range of enzyme materials and means for their incorporation into synthetic detergent compositions is also disclosed in WO 9307263 A and WO 9307260 A to Genencor International, WO 8908694 A to Novo, and U.S. 3,553,139, January 5, 1971 to McCarty et al. Enzymes are further disclosed in U.S. 4,101,457, Place et al, July 18, 1978, and in U.S. 4,507,219, Hughes, March 26, 1985. Enzyme materials useful for liquid detergent formulations, and their incorporation into such formulations, are disclosed in U.S. 4,261,868, Hora et al, April 14, 1981. Enzymes for use in detergents can be stabilised by various techniques. Enzyme stabilisation techniques are disclosed and exemplified in U.S. 3,600,319, August 17, 1971, Gedge et al, EP 199,405 and EP 200,586, October 29, 1986, Venegas. Enzyme stabilisation systems are also described, for example, in U.S. 3,519,570. A useful *Bacillus*, sp. AC13 giving proteases, xylanases and cellulases, is described in WO 9401532 A to Novo.

Color care benefits

Technologies which provide a type of color care benefit can also be included. Examples of these technologies are metallo catalysts for color maintenance. Such metallo catalysts are described in co-pending European Patent Application No. 92870181.2.

The Bleaching agent

The detergent compositions of the present invention can further include bleaching agents such as hydrogen peroxide, PB1, PB4 and percarbonate with a particle size of 400-800 microns. These bleaching agent components can include one or more oxygen bleaching agents and, depending upon the

bleaching agent chosen, one or more bleach activators. When present oxygen bleaching compounds will typically be present at levels of from about 1% to about 25%.

The bleaching agent component for use herein can be any of the bleaching agents useful for detergent compositions including oxygen bleaches as well as others known in the art. The bleaching agent suitable for the present invention can be an activated or non-activated bleaching agent.

One category of oxygen bleaching agent that can be used encompasses percarboxylic acid bleaching agents and salts thereof. Suitable examples of this class of agents include magnesium monoperoxyphthalate hexahydrate, the magnesium salt of meta-chloro perbenzoic acid, 4-nonylamino-4-oxoperoxybutyric acid and diperoxydodecanedioic acid. Such bleaching agents are disclosed in U.S. Patent 4,483,781, U.S. Patent Application 740,446, European Patent Application 0,133,354 and U.S. Patent 4,412,934. Highly preferred bleaching agents also include 6-nonylamino-6-oxoperoxycaproic acid as described in U.S. Patent 4,634,551.

Another category of bleaching agents that can be used encompasses the halogen bleaching agents. Examples of hypohalite bleaching agents, for example, include trichloro isocyanuric acid and the sodium and potassium dichloroisocyanurates and N-chloro and N-bromo alkane sulphonamides. Such materials are normally added at 0.5-10% by weight of the finished product, preferably 1-5% by weight.

The hydrogen peroxide releasing agents can be used in combination with bleach activators such as tetraacetylenediamine (TAED), nonanoyloxybenzene-sulfonate (NOBS, described in US 4,412,934), 3,5,5-trimethylhexanooloxybenzenesulfonate (ISONOBS, described in EP 120,591) or pentaacetylglucose (PAG) or Phenolsulfonate ester of N-nanoyl-6-aminocaproic acid (NACA-OBS, described in WO94/28106), which are perhydrolyzed to form a peracid as the active bleaching species, leading to improved bleaching effect. Also suitable activators are acylated citrate esters such as disclosed in Co-pending European Patent Application No. 91870207.7.

Useful bleaching agents, including peroxyacids and bleaching systems comprising bleach activators and peroxygen bleaching compounds for use in

detergent compositions according to the invention are described in our co-pending applications USSN 08/136,626, PCT/US95/07823, WO95/27772, WO95/27773, WO95/27774 and WO95/27775.

The hydrogen peroxide may also be present by adding an enzymatic system (i.e., an enzyme and a substrate therefore) which is capable of generating hydrogen peroxide at the beginning or during the washing and/or rinsing process. Such enzymatic systems are disclosed in EP Patent Application 91202655.6 filed October 9, 1991.

Metal-containing catalysts for use in bleach compositions, include cobalt-containing catalysts such as Pentaamine acetate cobalt(III) salts and manganese-containing catalysts such as those described in EPA 549 271; EPA 549 272; EPA 458 397; US 5,246,621; EPA 458 398; US 5,194,416 and US 5,114,611. Bleaching composition comprising a peroxy compound, a manganese-containing bleach catalyst and a chelating agent is described in the patent application No 94870206.3.

Bleaching agents other than oxygen bleaching agents are also known in the art and can be utilized herein. One type of non-oxygen bleaching agent of particular interest includes photoactivated bleaching agents such as the sulfonated zinc and/or aluminum phthalocyanines. These materials can be deposited upon the substrate during the washing process. Upon irradiation with light, in the presence of oxygen, such as by hanging clothes out to dry in the daylight, the sulfonated zinc phthalocyanine is activated and, consequently, the substrate is bleached. Preferred zinc phthalocyanine and a photoactivated bleaching process are described in U.S. Patent 4,033,718. Typically, detergent compositions will contain about 0.025% to about 1.25%, by weight, of sulfonated zinc phthalocyanine.

Builder system

The compositions according to the present invention may further comprise a builder system. Any conventional builder system is suitable for use herein including aluminosilicate materials, silicates, polycarboxylates, alkyl- or alkenyl-succinic acid and fatty acids, materials such as ethylenediamine tetraacetate,

diethylene triamine pentamethyleneacetat, metal ion sequestrants such as aminopolyphosphonates, particularly ethylenediamine tetramethylene phosphonic acid and diethylene triamine pentamethylenephosphonic acid. Phosphate builders can also be used herein.

Suitable builders can be an inorganic ion exchange material, commonly an inorganic hydrated aluminosilicate material, more particularly a hydrated synthetic zeolite such as hydrated zeolite A, X, B, HS or MAP.

Another suitable inorganic builder material is layered silicate, e.g. SKS-6 (Hoechst). SKS-6 is a crystalline layered silicate consisting of sodium silicate ($\text{Na}_2\text{Si}_2\text{O}_5$).

Suitable polycarboxylates containing one carboxy group include lactic acid, glycolic acid and ether derivatives thereof as disclosed in Belgian Patent Nos. 831,368, 821,369 and 821,370. Polycarboxylates containing two carboxy groups include the water-soluble salts of succinic acid, malonic acid, (ethylenedioxy) diacetic acid, maleic acid, diglycollic acid, tartaric acid, tartronic acid and fumaric acid, as well as the ether carboxylates described in German Offenlegungsschrift 2,446,686, and 2,446,687 and U.S. Patent No. 3,935,257 and the sulfinyl carboxylates described in Belgian Patent No. 840,623. Polycarboxylates containing three carboxy groups include, in particular, water-soluble citrates, aconitrates and citraconates as well as succinate derivatives such as the carboxymethyloxysuccinates described in British Patent No. 1,379,241, lactoxysuccinates described in Netherlands Application 7205873, and the oxypolycarboxylate materials such as 2-oxa-1,1,3-propane tricarboxylates described in British Patent No. 1,387,447.

Polycarboxylates containing four carboxy groups include oxydisuccinates disclosed in British Patent No. 1,261,829, 1,1,2,2-ethane tetracarboxylates, 1,1,3,3-propane tetracarboxylates and 1,1,2,3-propane tetracarboxylates. Polycarboxylates containing sulfo substituents include the sulfosuccinate derivatives disclosed in British Patent Nos. 1,398,421 and 1,398,422 and in U.S. Patent No. 3,936,448, and the sulfonated pyrolysed citrates described in British Patent No. 1,082,179, while polycarboxylates containing phosphone substituents are disclosed in British Patent No. 1,439,000.

Alicyclic and heterocyclic polycarboxylates include cyclopentane-cis,cis,cis-tetracarboxylates, cyclopentadienide pentacarboxylates, 2,3,4,5-tetrahydro-furan - cis, cis, cis-tetracarboxylates, 2,5-tetrahydro-furan -cis - dicarboxylates, 2,2,5,5-tetrahydrofuran - tetracarboxylates, 1,2,3,4,5,6-hexane - hexacarboxylates and and carboxymethyl derivatives of polyhydric alcohols such as sorbitol, mannitol and xylitol. Aromatic poly-carboxylates include m-llitic acid, pyromellitic acid and the phthalic acid derivatives disclosed in British Patent No. 1,425,343.

Of the above, the preferred polycarboxylates are hydroxycarboxylates containing up to three carboxy groups per molecule, more particularly citrates.

Preferred builder systems for use in the present compositions include a mixture of a water-insoluble aluminosilicate builder such as zeolite A or of a layered silicate (SKS-6), and a water-soluble carboxylate chelating agent such as citric acid. Preferred builder systems for use in liquid detergent compositions of the present inventions are soaps and polycarboxylates.

A suitable chelant for inclusion in the detergent compositions in accordance with the invention is ethylenediamine-N,N'-disuccinic acid (EDDS) or the alkali metal, alkaline earth metal, ammonium, or substituted ammonium salts thereof, or mixtures thereof. Preferred EDDS compounds are the free acid form and the sodium or magnesium salt thereof. Examples of such preferred sodium salts of EDDS include Na_2EDDS and Na_4EDDS . Examples of such preferred magnesium salts of EDDS include MgEDDS and Mg_2EDDS . The magnesium salts are the most preferred for inclusion in compositions in accordance with the invention.

Preferred builder systems include a mixture of a water-insoluble aluminosilicate builder such as zeolite A, and a watersoluble carboxylate chelating agent such as citric acid.

Other builder materials that can form part of the builder system for use in granular compositions include inorganic materials such as alkali metal carbonates, bicarbonates, silicates, and organic materials such as the organic phosphonates, amino polyalkylene phosphonates and amino polycarboxylates.

Other suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms. Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 20,000 to 70,000, especially about 40,000.

Detergency builder salts are normally included in amounts of from 5% to 80% by weight of the composition preferably from 10% to 70% and most usually from 30% to 60% by weight.

Suds suppressor

Another optional ingredient is a suds suppressor, exemplified by silicones, and silica-silicone mixtures. Silicones can be generally represented by alkylated polysiloxane materials while silica is normally used in finely divided forms exemplified by silica aerogels and xerogels and hydrophobic silicas of various types. These materials can be incorporated as particulates in which the suds suppressor is advantageously releasably incorporated in a water-soluble or water-dispersible, substantially non-surface-active detergent impermeable carrier. Alternatively the suds suppressor can be dissolved or dispersed in a liquid carrier and applied by spraying on to one or more of the other components.

A preferred silicone suds controlling agent is disclosed in Bartolotta et al. U.S. Patent 3 933 672. Other particularly useful suds suppressors are the self-emulsifying silicone suds suppressors, described in German Patent Application DTOS 2 646 126 published April 28, 1977. An example of such a compound is DC-544, commercially available from Dow Corning, which is a siloxane-glycol copolymer. Especially preferred suds controlling agent are the suds suppressor system comprising a mixture of silicone oils and 2-alkyl-alkanols. Suitable 2-alkyl-alkanols are 2-butyl-octanol which are commercially available under the trade name Isofol 12 R.

Such suds suppressor system are described in Co-pending European Patent application N 92870174.7 filed 10 November, 1992.

Especially preferred silicone suds controlling agents are described in Co-pending European Patent application N°92201649.8. Said compositions can

comprise a silicone/silica mixture in combination with fumed nonporous silica such as Aerosil®.

The suds suppressors described above are normally employed at levels of from 0.001% to 2% by weight of the composition, preferably from 0.01% to 1% by weight.

Others

Other components used in detergent compositions may be employed, such as soil-suspending agents, soil-release agents, optical brighteners, abrasives, bactericides, tarnish inhibitors, coloring agents, and/or encapsulated or non-encapsulated perfumes.

Especially suitable encapsulating materials are water soluble capsules which consist of a matrix of polysaccharide and polyhydroxy compounds such as described in GB 1,464,616.

Other suitable water soluble encapsulating materials comprise dextrans derived from ungelatinized starch acid-esters of substituted dicarboxylic acids such as described in US 3,455,838. These acid-ester dextrans are, preferably, prepared from such starches as waxy maize, waxy sorghum, sago, tapioca and potato. Suitable examples of said encapsulating materials include N-Lok manufactured by National Starch. The N-Lok encapsulating material consists of a modified maize starch and glucose. The starch is modified by adding monofunctional substituted groups such as octenyl succinic acid anhydride.

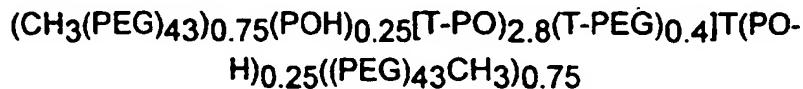
Antiredeposition and soil suspension agents suitable herein include cellulose derivatives such as methylcellulose, carboxymethylcellulose and hydroxyethylcellulose, and homo- or co-polymeric polycarboxylic acids or their salts. Polymers of this type include the polyacrylates and maleic anhydride-acrylic acid copolymers previously mentioned as builders, as well as copolymers of maleic anhydride with ethylene, methylvinyl ether or methacrylic acid, the maleic anhydride constituting at least 20 mole percent of the copolymer. These materials are normally used at levels of from 0.5% to 10% by weight, more

preferably from 0.75% to 8%, most preferably from 1% to 6% by weight of the composition.

Preferred optical brighteners are anionic in character, examples of which are disodium 4,4'-bis-(2-diethanolamino-4-anilino-s-triazin-6-ylamino)stilbene-2:2'-disulphonate, disodium 4, - 4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino-stilbene-2:2' - disulphonate, disodium 4,4' - bis-(2,4-dianilino-s-triazin-6-ylamino)stilbene-2:2' - disulphonate, monosodium 4',4" -bis-(2,4-dianilino-s-triazin-6-ylamino)stilbene-2-sulphonate, disodium 4,4' -bis-(2-anilino-4-(N-methyl-N-2-hydroxyethylamino)-s-triazin-6-ylamino)stilbene-2,2' - disulphonate, di-sodium 4,4' -bis-(4-phenyl-2,1,3-triazol-2-yl)-stilbene-2,2' disulphonate, di-sodium 4,4'bis(2-anilino-4-(1-methyl-2-hydroxyethylamino)-s-triazin-6-ylamino)stilbene-2,2'disulphonate, sodium 2(stilbyl-4"-(naphtho-1',2':4,5)-1,2,3 - triazole-2"-sulphonate and 4,4'-bis(2-sulphostyryl)biphenyl. Highly preferred brighteners are the specific brighteners of co-pending European Patent application No. 95201943.8.

Other useful polymeric materials are the polyethylene glycols, particularly those of molecular weight 1000-10000, more particularly 2000 to 8000 and most preferably about 4000. These are used at levels of from 0.20% to 5% more preferably from 0.25% to 2.5% by weight. These polymers and the previously mentioned homo- or co-polymeric polycarboxylate salts are valuable for improving whiteness maintenance, fabric ash deposition, and cleaning performance on clay, proteinaceous and oxidizable soils in the presence of transition metal impurities.

Soil release agents useful in compositions of the present invention are conventionally copolymers or terpolymers of terephthalic acid with ethylene glycol and/or propylene glycol units in various arrangements. Examples of such polymers are disclosed in the commonly assigned US Patent Nos. 4116885 and 4711730 and European Published Patent Application No. 0 272 033. A particular preferred polymer in accordance with EP-A-0 272 033 has the formula



where PEG is $-(OC_2H_4O)_n$, PO is (OC_3H_6O) and T is $(pcOC_6H_4CO)$.

Also very useful are modified polyesters as random copolymers of dimethyl terephthalate, dimethyl sulfoisophthalate, ethylene glycol and 1-2 propane diol, the end groups consisting primarily of sulphobenzoate and secondarily of mono esters of ethylene glycol and/or propane-diol. The target is to obtain a polymer capped at both end by sulphobenzoate groups, "primarily", in the present context most of said copolymers herein will be end-capped by sulphobenzoate groups. However, some copolymers will be less than fully capped, and therefore their end groups may consist of monoester of ethylene glycol and/or propane 1-2 diol, thereof consist "secondarily" of such species.

The selected polyesters herein contain about 46% by weight of dimethyl terephthalic acid, about 16% by weight of propane -1.2 diol, about 10% by weight ethylene glycol about 13% by weight of dimethyl sulfobenzoic acid and about 15% by weight of sulfoisophthalic acid, and have a molecular weight of about 3.000. The polyesters and their method of preparation are described in detail in EPA 311 342.

It is well known in the art that free chlorine in tap water rapidly deactivates the enzymes comprised in detergent compositions. Therefore, using chlorine scavenger such as perborate, ammonium sulfate, sodium sulphite or polyethyleneimine at a level above 0.1% by weight of total composition, in the formulas will provide improved through the wash stability of the detergent enzymes. Compositions comprising chlorine scavenger are described in the European patent application 92870018.6 filed January 31, 1992.

Alkoxylated polycarboxylates such as those prepared from polyacrylates are useful herein to provide additional grease removal performance. Such materials are described in WO 91/08281 and PCT 90/01815 at p. 4 et seq., incorporated herein by reference. Chemically, these materials comprise polyacrylates having one ethoxy side-chain per every 7-8 acrylate units. The side-chains are of the formula $-(CH_2CH_2O)_m(CH_2)_nCH_3$ wherein m is 2-3 and n is 6-12. The side-chains are ester-linked to the polyacrylate "backbone" to provide a "comb" polymer type structure. The molecular weight can vary, but is typically in the range of about 2000 to about 50,000. Such alkoxylated

polycarboxylates can comprise from about 0.05% to about 10%, by weight, of the compositions herein.

Softening agents

Fabric softening agents can also be incorporated into laundry detergent compositions in accordance with the present invention. These agents may be inorganic or organic in type. Inorganic softening agents are exemplified by the smectite clays disclosed in GB-A-1 400 898 and in USP 5,019,292. Organic fabric softening agents include the water insoluble tertiary amines as disclosed in GB-A1 514 276 and EP-B0 011 340 and their combination with mono C12-C14 quaternary ammonium salts are disclosed in EP-B-0 026 527 and EP-B-0 026 528 and di-long-chain amides as disclosed in EP-B-0 242 919. Other useful organic ingredients of fabric softening systems include high molecular weight polyethylene oxide materials as disclosed in EP-A-0 299 575 and 0 313 146.

Levels of smectite clay are normally in the range from 2% to 20%, more preferably from 5% to 15% by weight, with the material being added as a dry mixed component to the remainder of the formulation. Organic fabric softening agents such as the water-insoluble tertiary amines or dilong chain amide materials are incorporated at levels of from 0.5% to 5% by weight, normally from 1% to 3% by weight whilst the high molecular weight polyethylene oxide materials and the water soluble cationic materials are added at levels of from 0.1% to 2%, normally from 0.15% to 1.5% by weight. These materials are normally added to the spray dried portion of the composition, although in some instances it may be more convenient to add them as a dry mixed particulate, or spray them as molten liquid on to other solid components of the composition.

Dispersants

The detergent composition of the present invention can also contain dispersants : Suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 1,000 to 100,000.

Especially, copolymer of acrylate and methylacrylate such as the 480N having a molecular weight of 4000, at a level from 0.5-20% by weight of composition can be added in the detergent compositions of the present invention.

The compositions of the invention may contain a lime soap peptiser compound, which has a lime soap dispersing power (LSDP), as defined hereinafter of no more than 8, preferably no more than 7, most preferably no more than 6. The lime soap peptiser compound is preferably present at a level from 0% to 20% by weight.

A numerical measure of the effectiveness of a lime soap peptiser is given by the lime soap dispersant power (LSDP) which is determined using the lime soap dispersant test as described in an article by H.C. Borghetty and C.A. Bergman, J. Am. Oil. Chem. Soc., volume 27, pages 88-90, (1950). This lime soap dispersion test method is widely used by practitioners in this art field being referred to, for example, in the following review articles; W.N. Linfield, Surfactant science Series, Volume 7, page 3; W.N. Linfield, Tenside surf. det., volume 27, pages 159-163, (1990); and M.K. Nagarajan, W.F. Masler, Cosmetics and Toiletries, volume 104, pages 71-73, (1989). The LSDP is the % weight ratio of dispersing agent to sodium oleate required to disperse the lime soap deposits formed by 0.025g of sodium oleate in 30ml of water of 333ppm CaCO₃ (Ca:Mg=3:2) equivalent hardness.

Surfactants having good lime soap peptiser capability will include certain amine oxides, betaines, sulfobetaines, alkyl ethoxysulfates and ethoxylated alcohols.

Exemplary surfactants having a LSDP of no more than 8 for use in accord with the present invention include C₁₆-C₁₈ dimethyl amine oxide, C₁₂-C₁₈ alkyl ethoxysulfates with an average degree of ethoxylation of from 1-5, particularly C₁₂-C₁₅ alkyl ethoxysulfate surfactant with a degree of ethoxylation of amount 3

(LSDP=4), and the C₁₄-C₁₅ thoxylated alcohols with an average degree of ethoxylation of either 12 (LSDP=6) or 30, sold under the tradenames Lutensol A012 and Lutensol A030 respectively, by BASF GmbH.

Polymeric lime soap peptisers suitable for use herein are described in the article by M.K. Nagarajan, W.F. Masler, to be found in Cosmetics and Toiletries, volume 104, pages 71-73, (1989).

Hydrophobic bleaches such as 4-[N-octanoyl-6-amino hexanoyl]benzene sulfonate, 4-[N-nonenoyl-6-amino hexanoyl]benzene sulfonate, 4-[N-decanoyl-6-amino hexanoyl]benzene sulfonate and mixtures thereof; and nonanoyloxy benzene sulfonate together with hydrophilic / hydrophobic bleach formulations can also be used as lime soap peptisers compounds.

Dye transfer inhibition

The detergent compositions of the present invention can also include compounds for inhibiting dye transfer from one fabric to another of solubilized and suspended dyes encountered during fabric laundering operations involving colored fabrics.

Polymeric dye transfer inhibiting agents

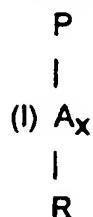
The detergent compositions according to the present invention also comprise from 0.001% to 10 %, preferably from 0.01% to 2%, more preferably from 0.05% to 1% by weight of polymeric dye transfer inhibiting agents. Said polymeric dye transfer inhibiting agents are normally incorporated into detergent compositions in order to inhibit the transfer of dyes from colored fabrics onto fabrics washed therewith. These polymers have the ability to complex or adsorb the fugitive dyes washed out of dyed fabrics before the dyes have the opportunity to become attached to other articles in the wash.

Especially suitable polymeric dye transfer inhibiting agents are polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinylpyrrolidone polymers, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof.

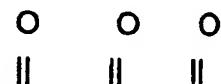
Addition of such polymers also enhances the performance of the enzymes according the invention.

a) Polyamine N-oxide polymers

The polyamine N-oxide polymers suitable for use contain units having the following structure formula :



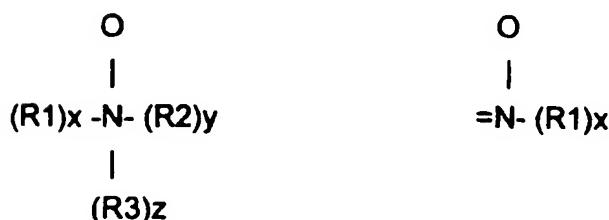
wherein P is a polymerisable unit, whereto the R-N-O group can be attached to or wherein the R-N-O group forms part of the polymerisable unit or a combination of both.



A is NC, CO, C, -O-, -S-, -N- ; x is 0 or 1;

R are aliphatic, ethoxylated aliphatics, aromatic, heterocyclic or alicyclic groups or any combination thereof whereto the nitrogen of the N-O group can be attached or wherein the nitrogen of the N-O group is part of these groups.

The N-O group can be represented by the following general structures :



wherein R1, R2, and R3 are aliphatic groups, aromatic, heterocyclic or alicyclic groups or combinations thereof, x or/and y or/and z is 0 or 1 and wherein the nitrogen of the N-O group can be attached or wherein the nitrogen of the N-O group forms part of these groups.

The N-O group can be part of the polymerisable unit (P) or can be attached to the polymeric backbone or a combination of both.

Suitable polyamine N-oxides wherein the N-O group forms part of the polymerisable unit comprise polyamine N-oxides wherein R is selected from aliphatic, aromatic, alicyclic or heterocyclic groups.

One class of said polyamine N-oxides comprises the group of polyamine N-oxides wherein the nitrogen of the N-O group forms part of the R-group. Preferred polyamine N-oxides are those wherein R is a heterocyclic group such as pyridine, pyrrole, imidazole, pyrrolidine, piperidine, quinoline, acridine and derivatives thereof.

Another class of said polyamine N-oxides comprises the group of polyamine N-oxides wherein the nitrogen of the N-O group is attached to the R-group.

Other suitable polyamine N-oxides are the polyamine oxides whereto the N-O group is attached to the polymerisable unit.

Preferred class of these polyamine N-oxides are the polyamine N-oxides having the general formula (I) wherein R is an aromatic, heterocyclic or alicyclic groups wherein the nitrogen of the N-O functional group is part of said R group.

Examples of these classes are polyamine oxides wherein R is a heterocyclic compound such as pyridine, pyrrole, imidazole and derivatives thereof.

Another preferred class of polyamine N-oxides are the polyamine oxides having the general formula (I) wherein R are aromatic, heterocyclic or alicyclic groups wherein the nitrogen of the N-O functional group is attached to said R groups.

Examples of these classes are polyamine oxides wherein R groups can be aromatic such as phenyl.

Any polymer backbone can be used as long as the amine oxide polymer formed is water-soluble and has dye transfer inhibiting properties. Examples of suitable polymeric backbones are polyvinyls, polyalkylenes, polyesters, polyethers, polyamide, polyimides, polyacrylates and mixtures thereof.

The amine N-oxide polymers of the present invention typically have a ratio of amine to the amine N-oxide of 10:1 to 1:1000000. However the amount of amine oxide groups present in the polyamine oxide polymer can be varied by appropriate copolymerization or by appropriate degree of N-oxidation. Preferably, the ratio of amine to amine N-oxide is from 2:3 to 1:1000000. More preferably

from 1:4 to 1:1000000, most preferably from 1:7 to 1:1000000. The polymers of the present invention actually encompass random or block copolymers where one monomer type is an amine N-oxide and the other monomer type is either an amine N-oxide or not. The amine oxide unit of the polyamine N-oxides has a $\text{PK}_a < 10$, preferably $\text{PK}_a < 7$, more preferred $\text{PK}_a < 6$.

The polyamine oxides can be obtained in almost any degree of polymerisation. The degree of polymerisation is not critical provided the material has the desired water-solubility and dye-suspending power.

Typically, the average molecular weight is within the range of 500 to 1000,000; preferably from 1,000 to 50,000, more preferably from 2,000 to 30,000, most preferably from 3,000 to 20,000.

b) Copolymers of N-vinylpyrrolidone and N-vinylimidazole

The N-vinylimidazole N-vinylpyrrolidone polymers used in the present invention have an average molecular weight range from 5,000-1,000,000, preferably from 5,000-200,000.

Highly preferred polymers for use in detergent compositions according to the present invention comprise a polymer selected from N-vinylimidazole N-vinylpyrrolidone copolymers wherein said polymer has an average molecular weight range from 5,000 to 50,000 more preferably from 8,000 to 30,000, most preferably from 10,000 to 20,000.

The average molecular weight range was determined by light scattering as described in Barth H.G. and Mays J.W. Chemical Analysis Vol 113, "Modern Methods of Polymer Characterization".

Highly preferred N-vinylimidazole N-vinylpyrrolidone copolymers have an average molecular weight range from 5,000 to 50,000; more preferably from 8,000 to 30,000; most preferably from 10,000 to 20,000.

The N-vinylimidazole N-vinylpyrrolidone copolymers characterized by having said average molecular weight range provide excellent dye transfer inhibiting properties while not adversely affecting the cleaning performance of detergent compositions formulated therewith.

The N-vinylimidazole N-vinylpyrrolidone copolymer of the present invention has a molar ratio of N-vinylimidazole to N-vinylpyrrolidone from 1 to 0.2, more preferably from 0.8 to 0.3, most preferably from 0.6 to 0.4.

c) Polyvinylpyrrolidone

The detergent compositions of the present invention may also utilize polyvinylpyrrolidone ("PVP") having an average molecular weight of from about 2,500 to about 400,000, preferably from about 5,000 to about 200,000, more preferably from about 5,000 to about 50,000, and most preferably from about 5,000 to about 15,000. Suitable polyvinylpyrrolidones are commercially available from ISP Corporation, New York, NY and Montreal, Canada under the product names PVP K-15 (viscosity molecular weight of 10,000), PVP K-30 (average molecular weight of 40,000), PVP K-60 (average molecular weight of 160,000), and PVP K-90 (average molecular weight of 360,000). Other suitable polyvinylpyrrolidones which are commercially available from BASF Cooperation include Sokalan HP 165 and Sokalan HP 12; polyvinylpyrrolidones known to persons skilled in the detergent field (see for example EP-A-262,897 and EP-A-256,696).

d) Polyvinyloxazolidone :

The detergent compositions of the present invention may also utilize polyvinyloxazolidone as a polymeric dye transfer inhibiting agent. Said polyvinyloxazolidones have an average molecular weight of from about 2,500 to about 400,000, preferably from about 5,000 to about 200,000, more preferably from about 5,000 to about 50,000, and most preferably from about 5,000 to about 15,000.

e) Polyvinylimidazole :

The detergent compositions of the present invention may also utilize polyvinylimidazole as polymeric dye transfer inhibiting agent. Said polyvinylimidazoles have an average about 2,500 to about 400,000, preferably from about 5,000 to about 200,000, more preferably from about 5,000 to about 50,000, and most preferably from about 5,000 to about 15,000.

f) Cross-linked polymers :

Cross-linked polymers are polymers whose backbone are interconnected to a certain degree; these links can be of chemical or physical nature, possibly with active groups in the backbone or on branches; cross-linked polymers have been described in the Journal of Polymer Science, volume 22, pages 1035-1039.

In one embodiment, the cross-linked polymers are made in such a way that they form a three-dimensional rigid structure, which can entrap dyes in the pores formed by the three-dimensional structure. In another embodiment, the cross-linked polymers entrap the dyes by swelling.

Such cross-linked polymers are described in the co-pending patent application 94870213.9

Method of washing

The compositions of the invention may be used in essentially any washing or cleaning methods, including soaking methods, pretreatment methods and methods with rinsing steps for which a separate rinse aid composition may be added.

The process described herein comprises contacting fabrics with a laundering solution in the usual manner and exemplified hereunder.

The process of the invention is conveniently carried out in the course of the cleaning process. The method of cleaning is preferably carried out at 5°C to 95°C, especially between 10°C and 60°C. The pH of the treatment solution is preferably from 7 to 11.

The following examples are meant to exemplify compositions of the present invention, but are not necessarily meant to limit or otherwise define the scope of the invention.

In the detergent compositions, the enzymes levels are expressed by pure enzyme by weight of the total composition and unless otherwise specified, the

detergent ingredients are expressed by weight of the total compositions. The abbreviated component identifications therein have the following meanings:

LAS	: Sodium linear C ₁₂ alkyl benzene sulphonate
TAS	: Sodium tallow alkyl sulphate
CXYAS	: Sodium C _{1X} - C _{1Y} alkyl sulfate
25EY	: A C ₁₂ -C ₁₅ predominantly linear primary alcohol condensed with an average of Y moles of ethylene oxide
CXYEZ	: A C _{1X} - C _{1Y} predominantly linear primary alcohol condensed with an average of Z moles of ethylene oxide
XYEZS	: C _{1X} - C _{1Y} sodium alkyl sulfate condensed with an average of Z moles of ethylene oxide per mole
QAS	: R ₂ .N ⁺ (CH ₃) ₂ (C ₂ H ₄ OH) with R ₂ = C ₁₂ -C ₁₄
Soap	: Sodium linear alkyl carboxylate derived from a 80/20 mixture of tallow and coconut oils.
TFAA	: C ₁₆ -C ₁₈ alkyl N-methyl glucamide.
TPKFA	: C ₁₂ -C ₁₄ tallow whole cut fatty acids.
DEQA	: Di-(taffoy-oxy-ethyl) dimethyl ammonium chloride.
SDASA	: 1:2 ratio of stearoyldimethyl amine:triple-pressed stearic acid.
Neodol 45-13	: C ₁₄ -C ₁₅ linear primary alcohol ethoxylate, sold by Shell Chemical CO.

Silicate	: Amorphous Sodium Silicate (SiO ₂ :Na ₂ O ratio = 2.0)
NaSKS-6	: Crystalline layered silicate of formula δ-Na ₂ Si ₂ O ₅ .
Carbonate	: Anhydrous sodium carbonate with a particle size between 200 μm and 900μm.
Bicarbonate	: Anhydrous sodium bicarbonate with a particle size between 400 μm and 1200μm.
STPP	: Anhydrous sodium tripolyphosphate
MAVAA	: Copolymer of 1:4 maleic/acrylic acid, average molecular weight about 80,000
Polyacrylate	: Polyacrylate homopolymer with an average molecular weight of 8,000 sold under the tradename PA30 by BASF GmbH
Zeolite A	: Hydrated Sodium Aluminosilicate of formula Na ₁₂ (AlO ₂ SiO ₂) ₁₂ . 27H ₂ O having a primary particle size in the range from 0.1 to 10 micrometers
Citrate	: Tri-sodium citrate dihydrate of activity 86.4% with a particle size distribution between 425 μm and 850 μm.
Citric	: Anhydrous citric acid
PB1	: Anhydrous sodium perborate monohydrate bleach, empirical formula NaBO ₂ .H ₂ O ₂
PB4	: Anhydrous sodium perborate tetrahydrate
Percarbonate	: Anhydrous sodium percarbonat bleach of empirical formula 2Na ₂ CO ₃ .3H ₂ O ₂

TAED	: Tetraacetyl ethylene diamine.
NOBS	: Nonanoyloxybenzene sulfonate in the form of the sodium salt.
Photoactivated Bleach	: Sulfonated zinc phtocyanine encapsulated in dextrin soluble polymer.
Antibody	: Rabbit anti-Alcalase provided by Novo Nordisk A/S at a dilution of 1:1000 (anti-Alcalase PPA 1619), Reference 28-901-03; anti-Savinase antibody PPA 1897, available from Novo Nordisk A/S and Anti-Protease B provided by Genencor.
Protease	: Proteolytic enzyme sold under the tradename Savinase and Alcalase by Novo Nordisk A/S, Maxapem, Maxacal and Maxatase by Gist-Brocades and proteases described in patents WO91/06637 and/or WO95/10591 and/or EP 251 446.
Amylase	: Amylolytic enzyme sold under the tradename Purafact Ox Am ^R described in WO 94/18314, WO96/05295 sold by Genencor; Termamyl [®] , Fungamyl [®] and Duramyl [®] , all available from Novo Nordisk A/S and those described in WO95/26397.
Lipase	: Lipolytic enzyme sold under the tradename Lipolase, Lipolase Ultra by Novo Nordisk A/S
Cellulase	: Cellulytic enzyme sold under the tradename Carezyme, Celluzyme and/or Endolase by Novo Nordisk A/S.
CMC	: Sodium carboxymethyl cellulose.

HEDP	: 1,1-hydroxyethane diphosphonic acid.
DETPMP	: Diethylene triamine penta (methylene phosphonic acid), marketed by Monsanto under the Trade name Dequest 2060.
PVNO	: Poly(4-vinylpyridine)-N-Oxide.
PVPVI	: Poly (4-vinylpyridine)-N-oxide/copolymer of vinyl-imidazole and vinyl-pyrrolidone.
Brightener 1	: Disodium 4,4'-bis(2-sulphostyryl)biphenyl.
Brightener 2	: Disodium 4,4'-bis(4-anilino-6-morpholino-1,3,5-triazin-2-yl) stilbene-2:2'-disulfonate.
Silicone antifoam	: Polydimethylsiloxane foam controller with siloxane-oxyalkylene copolymer as dispersing agent with a ratio of said foam controller to said dispersing agent of 10:1 to 100:1.
Granular Suds Suppressor	: 12% Silicone/silica, 18% stearyl alcohol, 70% starch in granular form
SRP 1	: Sulfobenzoyl end capped esters with oxyethylene oxy and terephthaloyl backbone.
SRP 2	: Diethoxylated poly (1,2 propylene terephthalate) short block polymer.
Sulphate	: Anhydrous sodium sulphate.
HMWPEO	: High molecular weight polyethylene oxide
PEG	: Polyethylene glycol.

Encapsulated perfume particles : Insoluble fragrance delivery technology utilising zeolite 13x, perfume and a dextrose/glycerin agglomerating binder.

Example 1 :

Antibodies' production:

Chickens were injected in the breast muscle with 1 ml of a 1 mg/ml Savinase® or Alcalase® emulsion. The proteolytic emulsion was prepared with a Freunds completed adjuvants (Freund and McDermott, 1942; Freund 1956) by intensively mixing an equal amount of Savinase® or Alcalase® solution (2 mg/ml) and Freunds complete adjuvant.

The immunisation scheme was completed with injections using a Freunds incomplete adjuvant and after a 4 weeks period, the eggs were collected during one week. The extraction of the antibodies from the eggs yolks was done according to the Polson extraction method as described in Immunological Investigation 19, 1990, pp 253-258.

Protease enzymatic inactivation :

A Savinase® or Alcalase® stock solution was prepared in a Tris Buffer (5x10⁻²M Tris, 25 mM NaCl, pH=8) at a concentration of 0.05% active enzyme. A Phosphate Buffer (0.05M KH₂PO₄, pH 6.8) contained the antibody (0.18 mg/ml). The interaction proteolytic enzyme (Final concentration 0.004%) / antibody was achieved in commercially available detergent solutions comprising Ariel liquid (0.8% in city water, pH 8.5) and Ariel Color Futur (0.8% in city water, pH 9.5) during 15 minutes at 40°C.

The proteolytic residual activity was measured according the method described in Delmar et al.(1979) Anal. Biochem. 99, pp316-320. Within 5 minutes the enzymatic activity of the protease was successfully blocked as shown in the table below.

Residual proteolytic activity

(in % after 5 minutes)

Protease	Ariel liquid	Ariel Color Futur
Savinase®	19	20
Alcalase®	1	1

Example 2

The following laundry detergent compositions were prepared in accord with the invention:

	I	II	III	IV	V	VI
LAS	8.0	8.0	8.0	8.0	8.0	8.0
C25E3	3.4	3.4	3.4	3.4	3.4	3.4
QAS	-	0.8	0.8	-	0.8	0.8
Zeolite A	18.1	18.1	18.1	18.1	18.1	18.1
Carbonate	13.0	13.0	13.0	27.0	27.0	27.0
Silicate	1.4	1.4	1.4	3.0	3.0	3.0
Sulfate	26.1	26.1	26.1	26.1	26.1	26.1
PB4	9.0	9.0	9.0	9.0	9.0	9.0
TAED	1.5	1.5	1.5	1.5	1.5	1.5
DETPMP	0.25	0.25	0.25	0.25	0.25	0.25
HEDP	0.3	0.3	0.3	0.3	0.3	0.3
Amylase	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
Protease	0.15	0.005	0.004	0.002	0.001	0.1
Antibody	0.5	0.01	0.1	0.01	0.001	1.0
MA/AA	0.3	0.3	0.3	0.3	0.3	0.3
CMC	0.2	0.2	0.2	0.2	0.2	0.2
Photoactivated bleach (ppm)	15	15	15	15	15	15
Brightener 1	0.09	0.09	0.09	0.09	0.09	0.09
Perfume	0.3	0.3	0.3	0.3	0.3	0.3
Silicon antifoam	0.5	0.5	0.5	0.5	0.5	0.5

Misc/minors to 100%

Density in g/litre	850	850	850	850	850	850
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Example 3

The following granular laundry detergent compositions of bulk density 750 g/litre were prepared in accord with the invention:

	I	II	III
LAS	5.25	5.61	4.76
TAS	1.25	1.86	1.57
C45AS	-	2.24	3.89
C25AE3S	-	0.76	1.18
C45E7	3.25	-	5.0
C25E3	-	5.5	-
QAS	0.8	2.0	2.0
STPP	19.7	-	-
Zeolite A	-	19.5	19.5
NaSKS-6/citric acid (79:21)	-	10.6	10.6
Carbonate	6.1	21.4	21.4
Bicarbonate	-	2.0	2.0
Silicate	6.8	-	-
Sodium sulfate	39.8	-	14.3
PB4	5.0	12.7	-
TAED	0.5	3.1	-
DETPMP	0.25	0.2	0.2
HEDP	-	0.3	0.3
Antibody	0.01	0.1	1.0
Protease	0.0026	0.0085	0.08
Lipase	0.003	0.003	0.003
Cellulase	0.00064	0.00064	0.00064
Amylase	0.0009	0.0009	0.0009
MA/AA	0.8	1.6	1.6
CMC	0.2	0.4	0.4

Photoactivated bleach (ppm)	15 ppm	27 ppm	27 ppm
Brightener 1	0.08	0.19	0.19
Brightener 2	-	0.04	0.04
Encapsulated perfume particles	0.3	0.3	0.3
Silicone antifoam	0.5	2.4	2.4
Minors/misc to 100%			

Example 4

The following detergent formulations, according to the present invention were prepared, where I is a phosphorus-containing detergent composition, II is a zeolite-containing detergent composition and III is a compact detergent composition:

	I	II	III
Blown Powder			
STPP	24.0	-	24.0
Zeolite A	-	24.0	-
C45AS	9.0	6.0	13.0
MA/AA	2.0	4.0	2.0
LAS	6.0	8.0	11.0
TAS	2.0	-	-
Silicate	7.0	3.0	3.0
CMC	1.0	1.0	0.5
Brightener 2	0.2	0.2	0.2
Soap	1.0	1.0	1.0
DETPMP	0.4	0.4	0.2
Spray On			
C45E7	2.5	2.5	2.0
C25E3	2.5	2.5	2.0
Silicone antifoam	0.3	0.3	0.3
Perfume	0.3	0.3	0.3
Dry additives			

Carbonate	6.0	13.0	15.0
PB4	18.0	18.0	10.0
PB1	4.0	4.0	0
TAED	3.0	3.0	1.0
Photoactivated bleach	0.02	0.02	0.02
Protease	0.01	0.002	0.05
Lipase	0.009	0.009	0.009
Amylase	0.002	0.003	0.001
Antibody	0.03	0.03	0.5
Dry mixed sodium sulfate	3.0	3.0	5.0
Balance (Moisture & Miscellaneous)	100.0	100.0	100.0
Density (g/litre)	630	670	670

Example 5

The following nil bleach-containing detergent formulations of particular use in the washing of colored clothing, according to the present invention were prepared:

	I	II	III
Blown Powder			
Zeolite A	15.0	15.0	-
Sodium sulfate	0.0	5.0	-
LAS	3.0	3.0	-
DETPMP	0.4	0.5	-
CMC	0.4	0.4	-
MA/AA	4.0	4.0	-
Agglomerates			
C45AS	-	-	11.0
LAS	6.0	5.0	-
TAS	3.0	2.0	-
Silicate	4.0	4.0	-
Zeolite A	10.0	15.0	13.0
CMC	-	-	0.5
MA/AA	-	-	2.0

	Carbonate	9.0	7.0	7.0
Spray On				
	Perfume	0.3	0.3	0.5
	C45E7	4.0	4.0	4.0
	C25E3	2.0	2.0	2.0
Dry additives				
	MA/AA	-	-	3.0
	NaSKS-6	-	-	12.0
	Citrate	10.0	-	8.0
	Bicarbonate	7.0	3.0	5.0
	Carbonate	8.0	5.0	7.0
	PVPVI/PVNO	0.5	0.5	0.5
	Lipase	0.009	0.009	0.009
	Amylase	0.005	0.005	0.005
	Cellulase	0.0014	0.0014	0.0014
	Protease	0.05	0.03	0.007
	Antibody	0.8	0.1	0.2
	Silicone antifoam	5.0	5.0	5.0
Dry additives				
	Sodium sulfate	0.0	9.0	0.0
	Balance (Moisture and Miscellaneous)	100.0	100.0	100.0
Density (g/litre)		700	700	700

Example 6

The following detergent formulations, according to the present invention were prepared:

	I	II	III	IV
LAS	20.0	14.0	24.0	22.0
QAS	0.7	1.0	-	0.7
TFAA	-	1.0	-	-
C25E5/C45E7	-	2.0	-	0.5
C45E3S	-	2.5	-	-

STPP	30.0	18.0	30.0	22.0
Silicate	9.0	5.0	10.0	8.0
Carbonate	13.0	7.5	-	5.0
Bicarbonate	-	7.5	-	-
DETPMP	0.7	1.0	-	-
SRP 1	0.3	0.2	-	0.1
MA/AA	2.0	1.5	2.0	1.0
CMC	0.8	0.4	0.4	0.2
Protease	0.08	0.002	0.02	0.005
Amylase	0.007	0.004	-	0.002
Lipase	0.004	0.002	0.004	0.002
Cellulase	0.0004	0.0001	-	-
Antibody	0.3	0.16	0.2	0.01
Photoactivated bleach (ppm)	70ppm	45ppm	-	10ppm
Brightener 1	0.2	0.2	0.08	0.2
PB1	6.0	2.0	-	-
NOBS	2.0	1.0	-	-
Balance (Moisture and Miscellaneous)	100	100	100	100

Example 7

The following detergent formulations, according to the present invention were prepared:

	I	II	III	IV
Blown Powder				
Zeolite A	30.0	22.0	6.0	6.7
Na SkS-6	-	-	-	3.3
Polycarboxylate	-	-	-	7.1
Sodium sulfate	19.0	5.0	7.0	-
MA/AA	3.0	3.0	6.0	-
LAS	14.0	12.0	22.0	21.5
C45AS	8.0	7.0	7.0	5.5

Cationic	-	-	-	1.0
Silicate	-	1.0	5.0	11.4
Soap	-	-	2.0	-
Brightener 1	0.2	0.2	0.2	-
Carbonate	8.0	16.0	20.0	10.0
DETPMP	-	0.4	0.4	-
Spray On				
C45E7	1.0	1.0	1.0	3.2
Dry additives				
PVPVI/PVNO	0.5	0.5	0.5	-
Antibody	0.2	0.09	0.15	0.05
Protease	0.053	0.01	0.005	0.01
Lipase	0.009	0.009	0.009	0.009
Amylase	0.0008	0.0008	0.0008	0.0008
Cellulase	0.0002	0.0002	0.0002	0.0002
NOBS	-	6.1	4.5	3.2
PB1	1.0	5.0	6.0	3.9
Sodium sulfate	-	6.0	-	to balance
Balance (Moisture and Miscellaneous)	100	100	100	

Example 8

The following high density and bleach-containing detergent formulations, according to the present invention were prepared:

	I	II	III
Blown Powder			
Zeolite A	15.0	15.0	15.0
Sodium sulfate	0.0	5.0	0.0
LAS	3.0	3.0	3.0
QAS	-	1.5	1.5
DETPMP	0.4	0.4	0.4
CMC	0.4	0.4	0.4
MA/AA	4.0	2.0	2.0

Agglomerates

LAS	5.0	5.0	5.0
TAS	2.0	2.0	1.0
Silicate	3.0	3.0	4.0
Zeolite A	8.0	8.0	8.0
Carbonate	8.0	8.0	4.0

Spray On

Perfume	0.3	0.3	0.3
C45E7	2.0	2.0	2.0
C25E3	2.0	-	-

Dry additives

Citrate	5.0	-	2.0
Bicarbonate	-	3.0	-
Carbonate	8.0	15.0	10.0
TAED	6.0	2.0	5.0
PB1	14.0	7.0	10.0

Polyethylene oxide of MW

5,000,000

Bentonite clay	-	-	10.0
Antibody	1.0	0.05	0.01
Protease	0.1	0.01	0.007
Lipase	0.009	0.009	0.009
Amylase	0.005	0.005	0.005
Cellulase	0.0014	0.0014	0.0014

Silicone antifoam

Dry additives

Sodium sulfate	0.0	3.0	0.0
Balance (Moisture and Miscellaneous)	100.0	100.0	100.0

Density (g/litre)

850

850

850

Example 9

The following high density detergent formulations, according to the present invention were prepared:

	I	II
Agglomerate		
C45AS	11.0	14.0
Zeolite A	15.0	6.0
Carbonate	4.0	8.0
MA/AA	4.0	2.0
CMC	0.5	0.5
DETPMP	0.4	0.4
Spray On		
C25E5	5.0	5.0
Perfume	0.5	0.5
Dry Adds		
HEDP	0.5	0.3
SKS 6	13.0	10.0
Citrate	3.0	1.0
TAED	5.0	7.0
Percarbonate	20.0	20.0
SRP 1	0.3	0.3
Antibody	0.05	0.015
Protease	0.014	0.005
Lipase	0.009	0.009
Cellulase	0.0014	0.0014
Amylase	0.005	0.005
Silicone antifoam	5.0	5.0
Brightener 1	0.2	0.2
Brightener 2	0.2	-
Balance (Moisture and Miscellaneous)	100	100
Density (g/litre)	850	850

Example 10

The following granular detergent formulations, according to the present invention were prepared:

	I	II	III	IV	V
LAS	21.0	25.0	18.0	18.0	-
Coco C12-14 AS	-	-	-	-	21.9
AE3S	-	-	1.5	1.5	2.3
Decyl dimethyl hydroxyethyl NH4+Cl	-	0.4	0.7	0.7	0.8
Nonionic	1.2	-	0.9	0.5	-
Coco C12-14 Fatty Alcohol	-	-	-	-	1.0
STPP	44.0	25.0	22.5	22.5	22.5
Zeolite A	7.0	10.0	-	-	8.0
MA/AA	-	-	0.9	0.9	-
SRP1	0.3	0.15	0.2	0.1	0.2
CMC	0.3	2.0	0.75	0.4	1.0
Carbonate	17.5	29.3	5.0	13.0	15.0
Silicate	2.0	-	7.6	7.9	-
Antibody	0.16	0.5	0.1	0.9	10.0
Protease	0.005	0.04	0.007	0.09	0.15
Amylase	-	0.004	0.004	-	.004
Lipase	0.003	0.003	0.003	-	-
Cellulase	-	0.001	0.001	.001	.001
NOBS	-	-	-	1.2	1.0
PB1	-	-	-	2.4	1.2
Diethylene triamine penta acetic acid	-	-	-	0.7	1.0
Diethylene triamine penta methyl phosphonic acid	-	-	0.6	-	-
Mg Sulfate	-	-	0.8	-	-
Photoactivated bleach	45	50 ppm	15	45 ppm	42
		ppm		ppm	ppm
Brightener 1	0.05	-	0.04	0.04	0.04
Brightener 2	0.1	0.3	0.05	0.13	0.13

Water and Minors up to 100%

Example 12

The following liquid detergent formulations, according to the present invention were prepared:

Antibody	0.1	0.01	10E-5	0.1	.001	10.0	0.01	.001
Protease	0.16	.002	.005	0.08	.008	0.1	0.01	.006
Lipase	-	.002	-	.0002	-	-	.003	.003
Amylase	.002	.002	-	.004	.002	-	.005	.005
Cellulase	-	-	-	.001	-	-	.002	.001
Boric acid	0.1	0.2	-	2.0	1.0	1.5	2.5	2.5
Na formate	-	-	1.0	-	-	-	-	-
Ca chloride	-	0.015	-	0.01	-	-	-	-
Bentonite clay	-	-	-	-	4.0	4.0	-	-
Suspending clay	-	-	-	-	0.6	0.3	-	-
SD3								
Balance Moisture and	100	100	100	100	100	100	100	100
Miscellaneous								

Example 12

Granular fabric cleaning compositions which provide "softening through the wash" capability were prepared in accord with the present invention :

	I	II
45AS	-	10.0
LAS	7.6	-
68AS	1.3	-
45E7	4.0	-
25E3	-	5.0
Coco-alkyl-dimethyl hydroxy-ethyl ammonium chloride	1.4	1.0
Citrate	5.0	3.0
Na-SKS-6	-	11.0
Zeolite A	15.0	15.0
MA/AA	4.0	4.0
DETPMP	0.4	0.4
PB1	15.0	-

Percarbonate	-	15.0
TAED	5.0	5.0
Smectite clay	10.0	10.0
HMWPEO	-	0.1
Antibody	0.075	0.1
Protease	0.02	0.006
Lipase	0.02	0.01
Amylase	0.03	0.005
Cellulase	0.001	-
Silicate	3.0	5.0
Carbonate	10.0	10.0
Granular suds suppressor	1.0	4.0
CMC	0.2	0.1
Water/minors	Up to 100%	

Example 13

The following rinse added fabric softener composition was prepared in accord with the present invention :

Softener active	20.0
Antibody	0.01
Protease	0.005
Cellulase	0.001
HCL	0.03
Antifoam agent	0.01
Blue dye	25ppm
CaCl ₂	0.20
Perfume	0.90
Water / minors	Up to 100%

Example 14

The following fabric softener composition was prepared in accord with the present invention :

	I	II	III
DEQA	2.60	19.00	-
SDASA	-	-	70.0
Stearic acid of IV=0	0.30	-	-
Neodol 45-13	-	-	13.0
Hydrochloride acid	0.02	0.02	-
Ethanol	-	-	1.0
PEG	-	0.60	-
Antibody	0.1	0.1	1.0
Protease	0.01	0.008	0.05
Perfume	1.00	1.00	0.75
Digeranyl Succinate	-	-	0.38
Silicone antifoam	0.01	0.01	-
Electrolyte	-	600ppm	-
Dye	100ppm	50ppm	0.01
Water and minors	100%	100%	-

Example 15

Syndet bar fabric cleaning compositions were prepared in accord with the present invention :

	I	II	III	IV
C26 AS	20.00	20.00	20.00	20.00
CFAA	5.0	5.0	5.0	5.0
LAS (C11-13)	10.0	10.0	10.0	10.0
Sodium carbonate	25.0	25.0	25.0	25.0
Sodium pyrophosphate	7.0	7.0	7.0	7.0
STPP	7.0	7.0	7.0	7.0

Z olite A	5.0	5.0	5.0	5.0
CMC	0.2	0.2	0.2	0.2
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.2
Coconut monethanolamide	5.0	5.0	5.0	5.0
Antibody	0.1	0.1	0.01	0.001
Amylase	0.01	-	0.01	-
Protease	0.08	0.01	0.005	0.001
Brightener, perfume	0.2	0.2	0.2	0.2
CaSO ₄	1.0	1.0	1.0	1.0
MgSO ₄	1.0	1.0	1.0	1.0
Water	4.0	4.0	4.0	4.0

Filler* : balance to 100%

*Can be selected from convenient materials such as CaCO₃, talc, clay (Kaolinite, Smectite), silicates, and the like.

Example 16

Detergent additives were prepared in accord with the present invention :

	I	II	III
LAS	-	-	5
STPP	30	30	-
Zeolite A	-	-	35
PB1	20	20	15
TAED	10	10	8
Protease	0.3	-	0.3
Amylase	0.1	-	-
Antibody	10	10	1.0

WHAT IS CLAIMED IS:

1. A detergent composition comprising a protease and a protease-directed antibody.
2. A detergent composition according to claim 1 wherein said protease-directed antibody is comprised at a level of from 10E-6% to 10E+1% by weight of total composition.
3. A detergent composition according to claims 1-2 wherein the protease is selected from serine proteases.
4. A detergent composition according to claims 1-3 wherein the protease is comprised at a level of from 0.0001% to 2%, preferably from 0.001% to 0.2%, more preferably from 0.005% to 0.1% of pure enzyme by weight of total composition.
5. A detergent composition according to claims 1-4 wherein the molecular ratio of protease-directed antibody to protease is equal or less than 100:1, preferably equal or less than 50:1.
6. A detergent composition according to claims 1-5 wherein said protease-directed antibody is a monoclonal antibody, preferably a fragment thereof.
7. A detergent composition according to claim 6 wherein the molecular ratio of said protease-directed antibody to protease is equal or less than 50:1, preferably equal or less than 20:1.
8. A detergent composition according to any of the preceding claims wherein said protease-directed antibody is incorporated into a release agent.
9. A detergent composition according to any of the preceding claims further comprising one or more components selected from anionic, nonionic, cationic, amphoteric and zwitterionic surfactants, builder, bleach system, suds suppressors, soil release polymer, lime soap dispersant, soil

suspension and anti-redeposition agents, smectite clays and/or mixtures thereof.

10. A detergent composition according to any of the preceding claims which is in the form of a liquid, granular, powder, gel, paste or bar.
11. A detergent additive comprising a protease-directed antibody.
12. A detergent additive according to claim 11 further comprising a protease.
13. Use of a protease-directed antibody in a detergent composition for controlling the proteolytic enzymatic activity.
14. Use of a protease-directed antibody according to claim 13 in a laundry detergent composition for providing fabric care benefits.

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/US 97/14288

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C11D3/386 C11D3/384

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 06574 A (NOVO NORDISK A/S) 16 May 1991 see page 5, line 13 - page 10, line 14 see claims ---	1,9,10
A	EP 0 298 654 A (HYBRISENS LTD.) 11 January 1989 see page 2, line 30 - page 4, line 20 see page 6, line 13 - line 16 see claims 1-8 ---	1
A	EP 0 628 624 A (THE PROCTER & GAMBLE CO.) 14 December 1994 see page 3, line 1 - line 17 see page 5, line 49 - page 14, line 23 see claims 1-7 ---	1,3,4,9, 10 -/-



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

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INTERNATIONAL SEARCH REPORT

Int'l Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 381 262 A (UNILEVER NV.) 8 August 1990 see page 4, line 3 - line 47; claims 1,6 ---	1,3,4,9, 10
A	WO 92 07067 A (NOVO NORDISK A/S) 30 April 1992 see page 7, line 28 - page 11, line 19 ---	1,3,4,9, 10
A	EP 0 345 750 A (OTSUKA PHARMACEUTICAL CO.) 13 December 1989 see claims 1,10 -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/14288

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9106574 A	16-05-91	AU 6634290 A EP 0494985 A JP 5503507 T		31-05-91 22-07-92 10-06-93
EP 0298654 A	11-01-89	AT 106250 T CA 1307737 A DE 3889783 D DE 3889783 T DK 375188 A ES 2056108 T JP 1138461 A		15-06-94 22-09-92 07-07-94 08-09-94 08-01-89 01-10-94 31-05-89
EP 0628624 A	14-12-94	EP 0653480 A EP 0587550 A EP 0635566 A CA 2176696 A CA 2176697 A CN 1138346 A EP 0736085 A WO 9517495 A WO 9517496 A AU 7974394 A WO 9513348 A AU 4545693 A CA 2140283 A CN 1084211 A JP 9501187 T MX 9304298 A WO 9402580 A US 5458809 A AU 7315094 A BR 9407201 A CA 2167373 A CN 1130399 A CZ 9600204 A HU 74035 A JP 9502745 T WO 9503382 A		17-05-95 16-03-94 25-01-95 29-06-95 29-06-95 18-12-96 09-10-96 29-06-95 29-06-95 29-05-95 18-05-95 14-02-94 03-02-94 23-03-94 04-02-97 31-01-95 03-02-94 17-10-95 20-02-95 17-09-96 02-02-95 04-09-96 17-07-96 28-10-96 18-03-97 02-02-95
EP 0381262 A	08-08-90	US 4959179 A		25-09-90

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l	Application No
PCT/US 97/14288	

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0381262 A		AU 619941 B AU 4880790 A CA 2008389 A,C DE 69023520 D DE 69023520 T ES 2081339 T JP 2276899 A JP 7065079 B US 5089163 A	06-02-92 02-08-90 30-07-90 21-12-95 18-04-96 01-03-96 13-11-90 12-07-95 18-02-92
WO 9207067 A	30-04-92	AT 122392 T DE 69109685 D DE 69109685 T EP 0552222 A ES 2073174 T JP 6503717 T US 5358865 A	15-05-95 14-06-95 04-01-96 28-07-93 01-08-95 28-04-94 25-10-94
EP 0345750 A	13-12-89	JP 1309686 A DE 68922340 D DE 68922340 T DK 277689 A ES 2070868 T US 5425942 A	14-12-89 01-06-95 26-10-95 09-12-89 16-06-95 20-06-95

